SEED BORNE DISEASES OF LINSEED 'FLAX' (*Linum usitatissimum* L.)

THESIS

Submitted for the degree of

DOCTOR OF PHILOSOPHY
IN SCIENCE

TO THE
UNIVERSITY OF ALLAHABAD

BY

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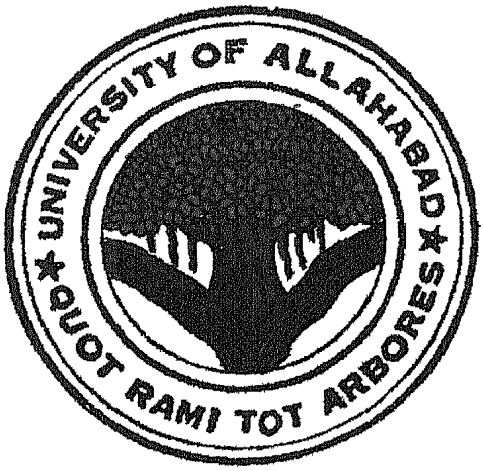

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Certificate

Certified that the thesis embodies results of original research work and study carried out under my supervision by Mr. Sunil Kumar Gupta, M. Sc. (Ag.) Agronomy.

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PREFACE

The thesis embodies research work on "**Seed-borne disease of Linseed** (*Linum Usitatissimum* L.)." The work carried out by me from 1999 to 2002 in the Bhargava Agricultural Laboratory, Garden and Farm of Botany Department, University of Allahabad.

The thesis embodies researches with studies on some seed-mycoflora isolated from Linseed seeds. The thesis contains work on isolation and pathological studies, effect of storage conditions, bio-chemical studies, studies of some isolated mycoflora with their respective host seeds and control studies.

The thesis is organised into ten chapters. First chapter deals with Introduction followed by Material and Methods (Chapter - 2). Isolation Studies and Pathological Studies are discussed in Chapter - 3 and Chapter - 4 respectively. Physiological and Ecological Studies are given in Chapter - 5. Chapter - 6 contains Storage Studies. Control and Inter-relationship Studies are discussed in Chapter - 7.

A detailed discussion on the basis of the present investigation and the summary are given in Chapter - 8 and Chapter - 9 respectively. The bibliography containing the references cited in the text is appended in the end (Chapter - 10). Abstract of the present work is also submitted along with the thesis separately.

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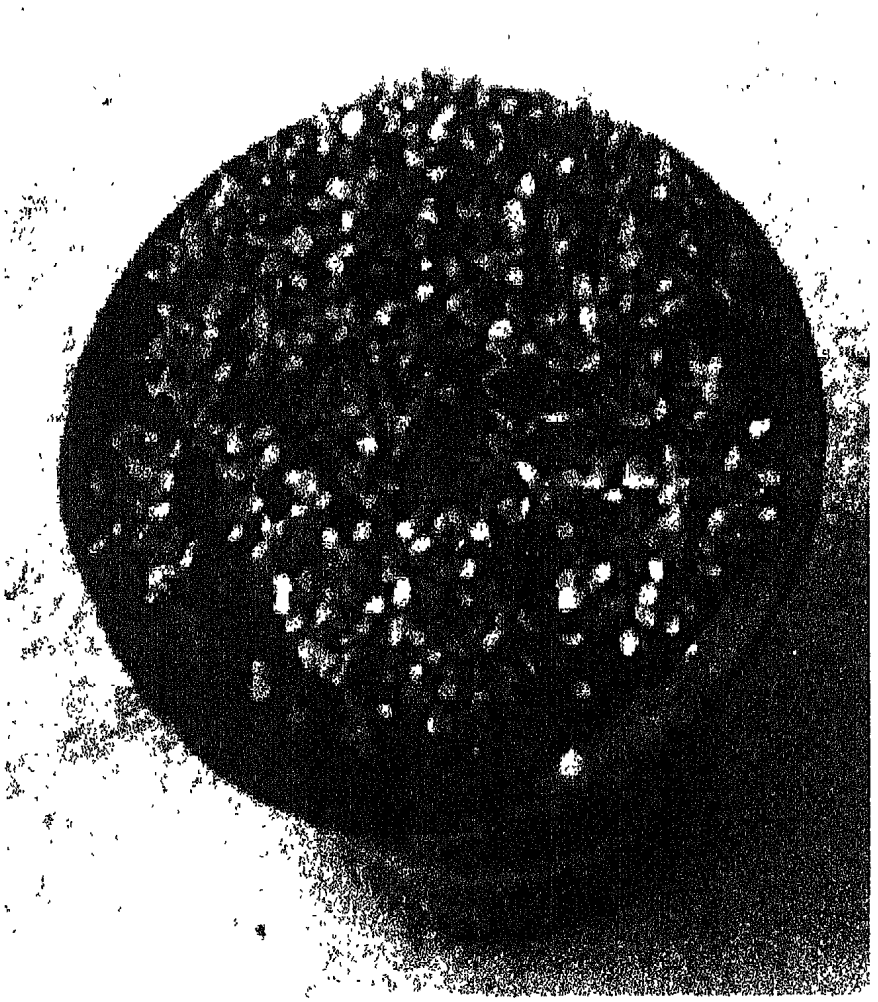
CONTENTS

CHAPTER – 1

INTRODUCTION



INTRODUCTION

"Every blade of grass is a study and to produce two where there was but one, is both a profit and a pleasure. And not grass alone, but soils, seeds and seasons; hedges, ditches and fences; draining, droughts and irrigations; ploughing, hoeing and harrowing; reaping, mowing and threshing; - saving crops, pests of crops, diseases of crops, and what will prevent or cure them; ..... the thousand things of which these are specimens, each a world of study within itself."

- Abraham Lincoln, 1859.

Oilseeds, the raw material for vegetable oils, occupy a significant place in India's national economy, next only to food grains, accounting for about 12 percent of the cultivated area and value of all agricultural produce. The nutritional significance of these oilseeds is quite vividly exemplified by the fact that it constitutes nearly 11 percent of the cost of living index (Kumar, 1982) with the present annual consumption of

about 4.5 kg per capita as against the world average consumption of 11 kg and 28 kg in affluent countries.

Linseed (*Linum usitatissimum* L.) is an oilseed crop cultivated in all over the world to produce linen and seeds for example, U. S. A., Argentina, Canada, India and Russia. In India, it grows in U. P., Bihar, Maharashtra and Punjab. About 4 million acres of land is cultivated in India and particularly in U. P. is one million-acre.

The linseed has developed many commercial and industrial uses. Its oil is used in production of paints, varnishes, oilcloths, soaps and leathers, glycerine, etc. Its straw is used in the preparation of different types of high value papers, such as cigarette, currency, bond, bible and airmail. Its cake is used for animal food and as fertilizer. Because of its importance and increasing popularity among cultivators the yield should be increased upto an extent that may exceed its present annual production. The increasing acreage is only possible when every factor is taken into consideration. The disease of linseed, however, is responsible in reduction of yield of linseed.

The good quality of fibres is also obtained from the stem of linseed, which are used for the manufacture of a variety of useful produce. After extraction of fibre, the remaining material contains properties of fibres. This material can be converted into pulp and utilised for the manufacture of high-grade writing paper and parchment paper. Recently this material has been found to be very useful for making a durable paper needed for currency notes.

Linseed is a winter season crop and requires moderate to cool temperature during crop period. In India, it is grown in about 2 million hectare with 526.8 thousand tonne production. Madhya Pradesh, Uttar Pradesh and Maharashtra are the leading states in acreage and production. These states together account for about 80 percent of the acreage and contribute 74 percent of the total production.

In India, the acreage, production and yield per hectare of linseed seeds have shown declining trend. This is due to increase in the cropping area, lack of technological application and protection of the crop from insect and diseases.

The linseed is subjected to attack by different pathogens such as fungi, bacteria, viruses and nematodes. Linseed suffers from many fungal diseases such as Rust - *Melampsora lini,* Stembreak and browning - *Polyspora lini* Laff , Anthracnose or seedling blight - *Colletotrichum lini* (*Pethyber ssp.* Laii), Alternaria blight - *Alternaria lini,* Powdery mildew - *Erysiphe spp.*, Wilt - *Fusarium lini (Bolley),* Plasma disease – *Sphaeralla lini colum (Wollenw),* Damping-off - *Pythium spp.*, Foot rot - *Phoma spp., Alternaria lini.*

First incidence of this disease was reported from Madhya Pradesh in 1923. The severe infection of the disease completely destroys the plants.

Seed-borne fungi in oilseeds may reduce the oil content, cause a change in its colour, induce an unpleasant odour and lead to hydrolytic rancidity (Sahasra Buddha and Kale, 1933; Wilson, 1947; Goodman and

Christenson, 1952; Ward and Diener, 1961; and Vidhyasekaran and Govindaswamy, 1963).

Seeds of oil crops are heavily affected both quantitatively and qualitatively by seed micro-organisms. In our country, very little attention has been paid towards seed fungal-flora of oil crops (Mohanty and Behre, 1958; Chavan, 1961; Joshi, 1961; Jain and Patel, 1969; Mishra *et al.*, 1969; Lambat *et al.*, 1969; Chohan, 1971; Kadiyan and Suryanarayana, 1971; Mishra *et al.*, 1972; Saharan and Gupta, 1972, 1973, 1974; Zimmer and Zimmermand, 1972; Mishra and Kanaujia, 1973; Agarwal and Singh, 1974; Ellis *et al.*, 1974; Petric, 1974; Chauhan and Sharma, 1975; Mathur and Kabeere, 1975; Rout, 1975; Singh *et al.*, 1975; Abraham *et al.*, 1976; Harrison and Perry, 1976; Irwin, 1976; Gupta *et al.*, 1977; Gaur and Ahamad, 1983; Law *et al.*, 1984; Vyas, 1984; Chandra *et al.*, 1985; Gupta *et al.*, 1985; Morsy *et al.*, 1985, Vaidehi and Lalita, 1985; and Jordon *et al.*, 1986).

Seeds play an important role in the dissemination of pathogens externally and internally, which affect the crop either at early or at late stages of growth (Goodman and Cristensen, 1952; Suryanarayana and Bhombe, 1961; Vidhyasekaran andGovindaswamy, 1968; Khare *et al.*, 1968; Noble and Richardson, 1968; Jain and Patel, 1969; Lambat *et al.*, 1969; Lalitha Kumari *et al.*, 1970; Raychaudhury, 1970; Ram Nath *et al.*, 1970; Neergaard, 1970; Kadian and Suryanarayana, 1971; Ram Nath and Lambat, 1971; Schneider *et al.*, 1971; Agarwal *et al.*, 1972; Agarwal and Singh, 1974; and Singh, 1974).

The above workers have also reported several pathogens responsible for reduction in germination percentage of seeds and an increase in the pre and post emergence rots of various crops. *Ascochyta imperfecta* casued alfalfa seeds to be light, dark and shrivelled with the production of spotted, distorted and necrotic seedlings (Karnkamp and Hemarie, 1952). *Alternaria brassicae* on rape caused a high percentage of shrunken seeds (Mc. Donald, 1959). Sackaton (1950) observed that flax seeds infected with *Suptoria linicola*, the cause of pasmo disease were smaller and lighter in weight than healthy seeds. From the review of literature, it is revealed that researches on seed mycoflora have greatly enhanced in the recent past in view of their importance as disease carriers, and deteriorating agents.

Fungi which contaminate the seeds of oil crops may be classified into two groups, i.e. field fungi and storage fungi, on the basis of stages at which invasion takes place. The field fungi are those which invade seeds while they are developing on the plants in the field until maturity but before they are harvested. It is, therefore, natural that seed flora of field fungi will vary from region to region depending upon climatic factors especially at seedling stage of the crop. Most congenial conditions for their invasion are high humidity and warmer temperatures. They may be externally seed-borne or deep-seated in the seeds.

Plant pathology is concerned with the health and productivity of growing plants. Disease losses are hazardous which can be minimised only by a continuous process of research and education. Each change in

variety of crop, each turn in the climatic cycle, and each regional shift in the growing of a crop bring new problems of health and disease. It is the responsibility of plant pathologists to evaluate and solve new disease problems, to train research investigators and extension specialists, to brief agricultural educators and country agents in applied phases of the science, and to work out practical procedures which growers can adopt to their needs.

Throughout the eighteenth century and the first half of the nineteenth century the taxonomy of plants was paramount and the dogma of constancy of species had a strong influence upon the trend of botanical thought. Moreover, the theory of spontaneous generation of microscopic forms and of the fungi generally held away. Parasitism was not an entirely new concept, for the parasitic phanerogams had been generally accepted as true parasites upon their hosts before this date, and De Bary in his first paper referred repeatedly to this fact in his argument for the acceptance of fungi as plant parasites.

Fungi are highly diverse and versatile organisms adopted to all kinds of environment. They constitute richest arrays of genera and species. These non-chlorophyllous plants agree with the green ones in having their protoplasm increased in cell walls. They however, differ essentially in their mode of nutrition. These are ubiquitous organisms found on earth, in water, in soil or in decomposing organic matter as saprophytes. They are also present in plants and animals as parasites or symbionts and play an essential part in the economy of nature.

The importance of seed-borne fungi is well recognised. They are responsible not only for the deterioration of the seeds in storage but also for a number of diseases appearing in the field. Further, many of them are responsible for poor seed germination and early mortality of seedlings. According to Christensen and Kaufmann (1963), fungi which contaminate the seeds may be classified into two groups, i.e. field fungi and storage fungi, on the basis of stages at which invasion takes place.

The field fungi are those which invade seeds while they are developing on the plants in the field until maturity but before they are harvested. it is, therefore, natural that seed flora of field fungi will vary from region to region depending upon climatic factors especially at seedling stage of the crop. Most congenial conditions for their invasion are high humidity and warmer temperature. They may be externally seed-borne or deep seated in the seeds. They may be located as sub-epidermal mycelia in the seeds. Field fungi may affect the quality and germineability of the seeds. Seed-borne infections of field fungi may also be responsible for diseases appearing in the field. Most of the field fungi, however, die if the seeds are stored properly, as low moisture is not conducive to their survival. Some common field fungi are *Alternaria, Curvularia, Cladosporium, Fusarium* and *Helminthosporium.*

The storage fungi are the ones which come in association with the seeds during transit and storage. These fungi grow on a variety of organic and inorganic substrata particularly decaying food products, dead plant materials, etc. They are abundantly present in the

atmosphere and thus serve as a source of contamination for seeds. Most of the storage fungi belong to genera *Aspergillus* and *Penicillium*. Storage fungi usually do not invade before harvest but they may be found on the seed in very low percentage nevertheless providing for the presence of inoculum of storage fungi. Moisture content and temperature play important role in the development of storage fungi in seeds. High humidity and high temperature favour their development. It has been shown that fungi associated with the stored seeds become active at the time of germination and cause seed rot and other losses.

The storage fungi may affect the seeds in various ways:

The storage fungi have tremendous influence on the grade and keeping quality of the seeds in storage. They deplete the reserve food material stored in the seed. They reduce the seedling vigour by producing hydrolytic enzymes, which affect the chemical constituents of the seed adversely. The storage fungi affect the seed viability adversely. The decrease in germination percentage of seeds during the storage is due to the invasion of the embryos by storage fungi. Storage fungi may cause discolouration of the embryo or of the whole seeds. Due to fungal invasion the quality of seed is very much affected and rancidity develops in the oil. Invasion by fungi also brings about biochemical changes. This is evident by increase in fatty acids, increase in reducing sugars, decrease in non-reducing sugars and increased respiration.

The growth of various fungi in stored seeds is accompanied by large amount of heat. The seeds get heated and sometimes the temperature reaches over 200°F in the portion of the bulk. This heating is mostly due to storage fungi because bacteria can only grow at higher moisture content. This finally results in decaying of seeds.

Many fungi, both field fungi and storage fungi, produce mycotoxins such as aflatoxin, etc. which are toxic, sometimes fatal to man and animals. In recent years, it has been shown that the mycotoxins produced in mouldy seeds not only inhibit the germination of seeds but also have severe after-effects on human beings on consumption.

Not only in foreign lands but also in India, due attention has been paid to the study of seed-borne fungi related to the diseases of growing plants of oil crops in fields. However, studies on the seed-borne fungi related to the spoilage of seeds in storage, poor seed germination and early mortality of seedlings are scanty.

For any variety or species of plant there is a set of environmental conditions, which favours its optimum development. In nature, however, such an ideal situation seldom exists, and in consequences every variety of plant is subjected to the vicissitudes of the environment. When one or more factors in the environment become particularly unfavourable, the development of the plant is altered in some way, and in comparison with plants growing in the usual range of environment it appears to have abnormal characteristics. Diseased

plants are distinguished by changes in their structure or physiological processes which are brought about by unfavourable environment or by one or another parasitic agency.

Temperature is one of the important external factor which influence biological systems. Fungi grow within a certain range of temperature, which varies, with the nature of the organism. Review on effect of temperature on fungal growth has been prepared by Togashi (1949), Hawker (1950), Cochrane (1958) and Deverall (1965). In the present study influence of temperature on growth and sporulation of above organisms has been observed.

The pH affects disease development through its effect on the pathogen or the host. The pathogens differ widely in their pH requirement and acidophilic and basophilic species are known in the same genus. Fungus has an optimum pH and also a minimum and maximum pH range for its growth. Suitability of the pH of a medium is therefore, of a considerable importance for proper growth and sporulation of fungi.

Fungi like higher plants need carbon, nitrogen, hydrogen, oxygen, phosphorus, sulphur, potassium, magnesium, traces of certain metabolites and growth promoting substances for their growth and reproduction. Among them carbon plays a key role. Like carbon, nitrogen is also employed both for structural and functional purposes by fungi. Nitrogen nutrition of pathogenic fungi has been well illustrated by Wolf *et al.* (1950); Lilly and Barnett (1951); Cochrane

(1958) and Haarris and Taber (1970). Of the organic sources of nitrogen the acids have been generally considered to be the most common choice for fungal growth and reproduction. It was therefore, considered desirable to study the nitrogen metabolism of the fungi under investigation. Carbon and Nitrogen levels in the culture medium have been known to influence the growth and sporulation of the fungi. Generally, it has been reported that lower C/N ratio induced sporulation under laboratory conditions (Hasija, 1970). In the present investigation an attempt has been made to study the effect of C/N ratio on the growth and sporulation of the organisms under investigation.

Soil is supposed to be a medium as a complex environment where fungi including other micro-organisms almost compete for their existence.

Planning for disease control involves strategy and tactics and demands knowledge of the chemical use for controlling plant disease. Interest in use of fungicides in plant disease control has vastly increased. Their use in controlling various diseases of fruits and fruit crops have been suggested by numerous workers in the last few years, viz. Daines and Snee (1969); Gutter (1970); Spalding and Reeder (1972); Bhargava and Singh (1975); Quimo and Quimo (1974), Pathak and Shekhawat (1977) and Tsai (1978). In order to economise and avoid wastage of fungicides, it is pre-requisite to evaluate the fungicides in laboratory before testing them on fruits. It not only save time but also prevents the wastage of ineffective ones. In the present study, therefore,

an attempt has been made to evolve suitable methods for controlling the diseases under investigation.

A seed-borne pathogen may or may not be seed transmitted. It is a reasonable assumption that seed transmission is established scientifically for a pathogen only when any other means of transmission is excluded. Establishment of seed infection is much more complicated. Several factors are responsible viz. physiological conditions pertaining to the pathogens and the host and weather conditions, for including infection. The establishment and development of infection within the seedling are subsequent adult plant is the last decisive link in the process of seed transmission.

It is a well-known fact that some fungi secrete mycotoxins, which are responsible for the reduction in seed germination and cause wilting, stunting of the seedlings and also effect the seed viability (Suryanarayana and Bhombe, 1961 and Dwivedi and Tandon, 1975). These not only affect seed viability, but are also harmful and dangerous for animal and human beings, if consumed. *Aspergillus flavus* group of organism and several species of *Penicillium* are known to produce toxins in groundnut and cottonseeds. Some species of *Fusarium* such as *F. graminearum* causing blight of wheat, barley and rye, which are known to produce toxic symptoms on consumption of infected grains.

Infected grains due to *F. poae* and *f. sporotrichoides* caused a fatal human toxicoses during 1942 - 43 in the U. S. S. R. Similarly Ergots

of Pearl millet rye and other forage grasses can cause well known ergot poisoning in man and domestic animals.

The variation in the number and type of fungal flora associated with different types of seed, differs due to variation in the physio-chemical nature of the seed, agricultural operations, storage and climatological conditions of the locality under sampling.

Decrease in viability of seeds due to storage affects the fungal population adversely. Fresh seeds are associated with diverse type of field and storage fungi which grow and multiply at the cost of the seed as long as the food and moisture supply are adequate and temperature is not unfavourable. With lapse of time, the micro-ecological conditions of the seed is disturbed and the environmental set up becomes less favourable to a number of fungi due to depletion in food level and low moisture supply. Moisture content of seeds may vary greatly within a bulk.

The inter-relationship between seed and seed-fungi is very complex. The seeds in turn also play some part in regulating the physiological set up of the fungi associated with them. Seed-coat leachates have been reported to cause inhibitory effect on certain seed-fungi (Ark and Thomson, 1958; Srivastava and Mishra, 1971 and Mishra and Kanaujia, 1973).

The germ damaged by storage fungi may end up as dark particles in the final production, impart undesirable flavours to food and they break down the constituents of seeds and absorb them. The fats are

broken down into fatty acids and starch into carbon dioxide and alcohol.

Consumers are interested in food values of the seeds while the stockists are mainly interested in the storability of the seeds. The storability is largely dependent upon the chemical changes which take place in the seeds during storage and is governed by temperature, humidity, aeration and microbial activities. It is said that without an ample store of seeds there can be no national treasure or no future for the nation.

The present problem was undertaken as an integral part of a comprehensive plan of experimental investigation, employing various organised studies for the furtherance of scientific knowledge in this important and complex field of research. The investigations were conducted with recognised methodology.

In the present investigation an attempt has been made to study the fungal flora of seeds of some unknown and known varieties of linseed crops. An attempt has also been made to isolate fungi from seeds of different age as well as from seeds stored at different length of time.

Fungi were isolated, cultured, purified, maintained and identified. Effect of moisture content present in different seeds of oil crops and percentage of seeds contaminated with fungal flora has also been observed. Pathogenicity tests have been carried out with these organisms. Symptoms if produced, recorded on their respective hosts.

Fungicides have been evaluated in the laboratory against some pathogenic forms and the successful ones have been further tried in the epiphytic field conditions. An attempt has also been made to investigate the inter-relationship between certain linseed crops and their seed borne fungi. Some biochemical studies have been carried out, which included the effect of some seed borne fungi on oil contents of seeds.

The informations generated from the present studies are expected to near the goal for economic and stable control measures in addition to finding resistant varieties.

CHAPTER – 2

MATERIALS AND METHODS

MATERIALS AND METHODS

During the year 1999 - 2000 a survey for prevalence and severity of seed borne diseases of Linseed in different Agro-climatic conditions, such as Allahabad, Banda, Hamirpur, Mahoba and its adjacent regions was made. Various seed samples of linseed crop were collected. Blotter and the Agar plate methods were used for detection of seed borne and surface fungi.

Various seed samples of linseed crop were collected from I.A.R.I., New Delhi; Allahabad Agricultural Institute, Allahabad; G. B. Pant University of Agriculture and Technology, Pantnagar and from local markets. Fungi was isolated, purified and maintained on malt extract and Potato Dextrose Agar media, for further examination and pathogenicity tests. Morphological studies were carried out and identifications were made.

The seeds were brought to laboratory and stored in sterilized glass containers to study the surface fungal flora. Seeds were directly placed on petridishes containing malt extract agar medium and on moist sterilized blotting paper. 100 seeds of linseed crop were taken in each case. To study the internal seed fungal flora, the seeds were dipped in 0.1% mercuric chloride for 2 - 3 minutes, rinsed several times in sterilized distilled water and were placed similarly as above on

petridishes containing malt extract agar on moist sterilized blotting paper.

Petridishes containing seeds were incubated for 7 to 10 days at 25°C ± 1°C. Any fungal growth observed on seeds during this period was picked up and subsequently grown on malt extract or P. D. A. on Asthana and Hawkers medium 'A' for further studies. The fresh stock of cultures was maintained by sub-culturing the fungus at regular intervals of three weeks on potato Dextrose Agar medium and also where it was necessary, single spore cultures of the fungi were prepared with the help of dummy culture objective. The following media were used for isolation and other studies:

1. Malt extract Agar Medium (Raper and Thom, 1949)

Agar	25.0 gm
Malt extract (Difco)	20.0 gm
Dextrose	20.0 gm
Peptone	1.0 gm
Water (distilled)	1000 ml

2. Potato dextrose Agar medium (Riker and Riker, 1936)

Agar	17.0 gm
Potato	200.0 gm
Dextrose	20.0 gm

Water	1000 ml
pH	6.0 – 6.5

200 gm of potato was washed, cut into small pieces and boiled for half an hour in 500 ml distilled water and then filtered through muslin cloth. 20 gm of dextrose was added to it and final volume was made-up to 1000 ml.

3. Asthana and Hawker's medium 'A'

Glucose	5.00 gm
KNO_3	3.50 gm
Potassium dihydrogen phosphate ($CCKH_2PO_4$)	1.75 gm
Agar	20.00 gm
Magnesium sulphate ($MgSO_4\ 7H_2O$)	0.75 gm
Distilled water	1000 ml

4. Oat meal Agar

Oat meal	50.00 gm
Agar	20.00 gm
Distilled water	1000 ml

solution of malachite green (50 mg/litre), Captan (100 gm/litre) and Dicryaticin (0.75 mg/litre) were added. The medium was lightly cooled and 20 ml was poured into each plate to solidify. 50 mg of the soil was sprinkled on solidified surface of the medium and the Petridishes were incubated at 25°C ± 1°C for 3 - 5 days.

Diseased roots of Linseed crop were washed with water, cut into small bits, surface sterilized with 0.1 percent mercuric chloride solution and finally washed with several changes of sterilized water. The sterilized root bits were placed into sterilized Potato Dextrose Agar medium (Peeled potato 250 gm, agar bag, dextrose 20 gm, distilled water 1000 ml). Previously poured into sterilized petridishes by half plate method. The plates were incubated at room temperature (28°C ± 2°C). Profuse growth of the mycelium was seen within seven days.

The isolated fungi were artificially inoculated in Pot soil through sand maize medium at 20 percent (W/W) when Linseed seedlings planted in these pots and field were 45 days old. The plants exhibited wilt symptoms in a period of 25 days. The pathogen was re-isolated from these artificially inoculated plants and maintained on oats slants for further studies.

50 gm of leaf was washed, chopped and crushed using mixie with 100 ml of distilled water and filtered through muslin cloth. Then the filtrates were used for inhibition zone technique by paper disc method and poisoned food technique.

In this method, distilled water at 70°C was used instead of cold distilled water. Then these extracts were used for inhibition zone technique by paper disc method and poisoned food technique. The leaf material used for cold distilled water extraction alone was used for hot distilled water extraction at the same concentration.

The method followed by Eben and Keil (1950) was slightly modified and carried out. Sterilized filter paper discs (10 mm) were soaked in cold and hot distilled water plant extracts and 50 percent concentration. In each of the Petriplates two treated discs were placed at the centre on potato Dextrose Agar medium. Suitable controls were maintained by placing the discs soaked in sterile water. The mycelial discs (9 mm) were kept at three places on the periphery of the petriplates at equal distance. Each treatment was replicated three times and incubated at room temperature (28°C ± 2°C) for 24 hours.

The effect of plant extracts on the growth of the pathogens was studied by poisoned food technique followed by Grower and Moores (1962). 50 gm of different plant leaf materials extracted in 100 ml of hot and cold distilled water were used. 5 ml of these extracts were taken to incorporate into 50 ml of Potato Dextrose Agar medium and autoclaved for 20 minutes at 1.41 kg/cm^2 pressure. The plant extract incorporated medium at 5 percent was poured into the sterilized petriplates and allowed to cool. The plates were inoculated with uniform discs of 9 mm diameter from 3-day old culture grown on Potato Dextrose Agar medium. The diameter of colony growth was recorded after 24 hours and the mean inhibition percentage worked out. The inoculated

petriplates were incubated at room temperature (28°C ± 2°C) for 24 hours with suitable control.

The treated and sterilized water soaked seeds were kept for germination in roll towel and each treatment was replicated three times. The seeds were kept for germination at 25°C ± 5°C and 95 ±2 percent relative humidity.

Plant extracts tested under roll towel method were applied on the Linseed crop seeds at 50 percent concentration for 6 hours and then shade dried for 2 hours. Pathogen multiplied on sand maize medium (19 : 1 ratio) was used for the inoculation of un-sterilised soil. The inoculum was mixed at the rate of 5 percent to the soil and it was incorporated one day before sowing. Each treatment was replicated three times. One hundred seeds were used for each treatment and the observations were recorded. Similar experiment was also conducted to assess the efficacy of storage seed treatment on the seedling mortality.

Pathogen multiplied on sand maize medium was incorporated into the un-sterilised soil one day before sowing the seeds at the rate of 5 gm per 100 gm of soil in earthen pots. Linseed seeds were sown in inoculated soil and the plant extracts used for seed treatment were also used for soil drenching at 25 percent concentration immediately after sowing. Drenching the soil was given upto 5 cm depth. Seeds sown in pathogen inoculated and uninoculated control were also drenched with tap water. Each treatment was replicated three times. One hundred seeds were used for each treatment.

For this study, eleven fungicides viz., Agroson GN (Tolyl mercury acetate), Agallol - 3 (2-methyl 1-ethyl mercury chloride), Blitox (Copper oxychloride), Ceresan dry (Phenyl mercyry acetate), Ceresan wet (Ethoxy methyl mercury chloride), Captan (N-trichloro methylthio-4-cyclohexen-1, 2-dicarboximide), Dithane M-45 (Zinc-manganese ethylenebisdithio carbamate), Dithane Z-78 (Zinc ethylene bisdithio carbamate 75 percent), Sulphur dust (elemental sulphur), Thiram (Tetra methyl thirum disulphide), Tillex (Ethyl-mercury chloride) and an antibiotic Streptomycin (streptomycin sulphate) were used.

The seeds were soaked in different concentrations of fungicides (500, 1000 and 1500 ppm) for 15 - 20 minutes and stored for 48 hours. The treated seeds were than plated on PDA plates as well as on sterilized moist blotters. The observations for seed germination, seedling vigour and seed mycoflora were taken after 7 - 10 days.

Thiram, which proved to be the most efficacious fungicide, was used in this study. Linseed seeds were treated with 0.4 percent concentration of Thiram and were stored in sterilized tight screwed glass containers under laboratory condition (20 - 28°C). In control series, untreated seeds were used. After six months of storage, germinability and the mycoflora of the stored seeds were determined.

CHAPTER – 3

ISOLATION STUDIES

ISOLATION STUDIES

Seeds of linseed crop usually get infected through pods and fruits. The site of infection in the fleshy cotyledons, which form a rich nutritious base for the seedlings as well as for pathogens may essentially play a key role in transmission of these pathogens. According to Neergaard (1977), a disease is borne in the seed, in the sense that potentially it is brought forth or given support by the seed. An investigation was thus undertaken to study in detail, the seed borne diseases on linseed crop.

Seeds of linseed crop were collected from various places and fungi were isolated, purified and maintained on media as mentioned in chapter - 2 and results recorded in Table - 1.

A similar study was also carried out with different varieties of linseed crop and the results are tabulated in Table - 2.

To isolate fungal flora from cotyledons, treated seeds were soaked at different intervals in sterilized water. The seed coat was removed and cotyledons were placed on petridishes containing malt extract agar media.

In linseed crop seeds, maximum association of *Fusarium* species was also observed on and in the treated and untreated seeds. The other

fungi which were isolated from seed surface includes species of *Aspergillus, Alternaria, Curvularia, Chaetomium, Rhizopus, Macrophomina* and *Penicillium* while in treated seeds species of *Fusarium, Alternaria, Curvularia, Chaetomium* could be detected. Twelve species were internally seed borne and other externally seed borne.

The fungi isolated from known varieties of seeds of Linseed were similar to those isolated from composite samples of unknown varieties.

Table - 1 Fungi isolated from seeds of linseed 'Flax' (*Linum usitatissimum* L.)

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Macrophomina phaseolina (Tassai) Gold	*Aspergillus niger* and *Alternaria alternata*
* *Alternaria* state of *Pleospora infectoria* Fuckel	Fusarium equiseti
* *Aspergillus flavus* Link	*Aspergillus flavus*
**Fusarium equiseti* (Corda) Sacc.	*Fusarium solani*
Fusarium oxysporum Schlecht.	
Aspergillus terreus Thom.	
**Alternaria alternata* (Fr.) Keisslerr	
Aspergillus niger Van tieghem.	
Aspergillus fumigatus Fresen.	
Chaetomium arcuatum (Rai and Tiwari)	
Curvularia lunata (Wakker) Beedizn.	
Drechslera spp.	
Penicillium javanicum Van bemya.	

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Rhizopus arrhisus Fischer	
* *Fusarium solani* (Mart.)	
* *Fusarium semilectum* Berk and Curt.	
* *Fusarium moniloforme* Sheld.	
Cladosporium oxysporum Berk and Curt.	
Cunninghamella achinulata	
* *Alternaria circinans*	
* *Alternaria tencissima*	*A. tencissima*
Aspergillus flavus Link	
Aspergillus niger	*Aspergillus flavus*
Ascochyta mycosphaerella rabiei	
Botrytis cinerea	*Botrytis cinerea*
Colletotrichum dematium	*Colletotrichum dematium*
* *Curvularia verruculara*	
Cochiliobalus spicifer	

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Fusarium othocarpus	*Fusarium othocarpus*
* *Fusarium sp.*	
Operculell paduicki	*operculell paduicki*
Phyllosticta rabiei	*Phyllosticta rabiei*
Penicillium globosum	*Penicillium globosum*
Rhizoctonia beticola	*Rhizoctonia beticola*
Rhizopus arrhizus	*Rhizopus arrhizus*
Scieritinia scleratiorum	*Scieritinia scleratiorum*
	Sclerotium rolfsii
Stimphylium sarciniforme	*Stimphylium sarciniforme*
Stachybatrys atra	*Stachybatrys atra*
Uromyces ciceresarietini	*Uromycesciceresarietini*

* Denotes internally seed borne

Table - 2 Fungi isolated from some varietal samples of linseed 'Flax' (*Linum usitatissimum* L.)

Varieties	No. of seeds taken	Seed Germ (%)	Percentage incidence of mycoflora								
			A	B	C	D	E	F	G	H	I
Heera	UT 100	52	68	73	-	-	-	32	-	-	-
	T 100	81	36	48	-	-	-	19	-	-	-
Neelam	UT 100	58	52	68	-	-	-	11	-	-	-
	T 100	85	21	43	-	-	-	7	-	-	-
Mukta	UT 100	53	-	61	-	-	-	57	9	21	13
	T 100	83	-	38	-	-	-	23	4	17	5
Pantnagar	UT 100	66	38	23	8	-	32	36	21	-	-
	T 100	92	21	11	2	-	17	19	8	-	-
N - 55	UT 100	68	10	78	-	34	52	-	-	-	-
	T 100	90	4	42	-	31	27	-	-	-	-

Where UT = Untreated seeds

T = Treated seeds

A = *Alternaria spp.*

B = *Aspergillus spp.*

C = *Curvularia spp.*

D = *Penicillium spp.*

E = *Rhizopus spp.*

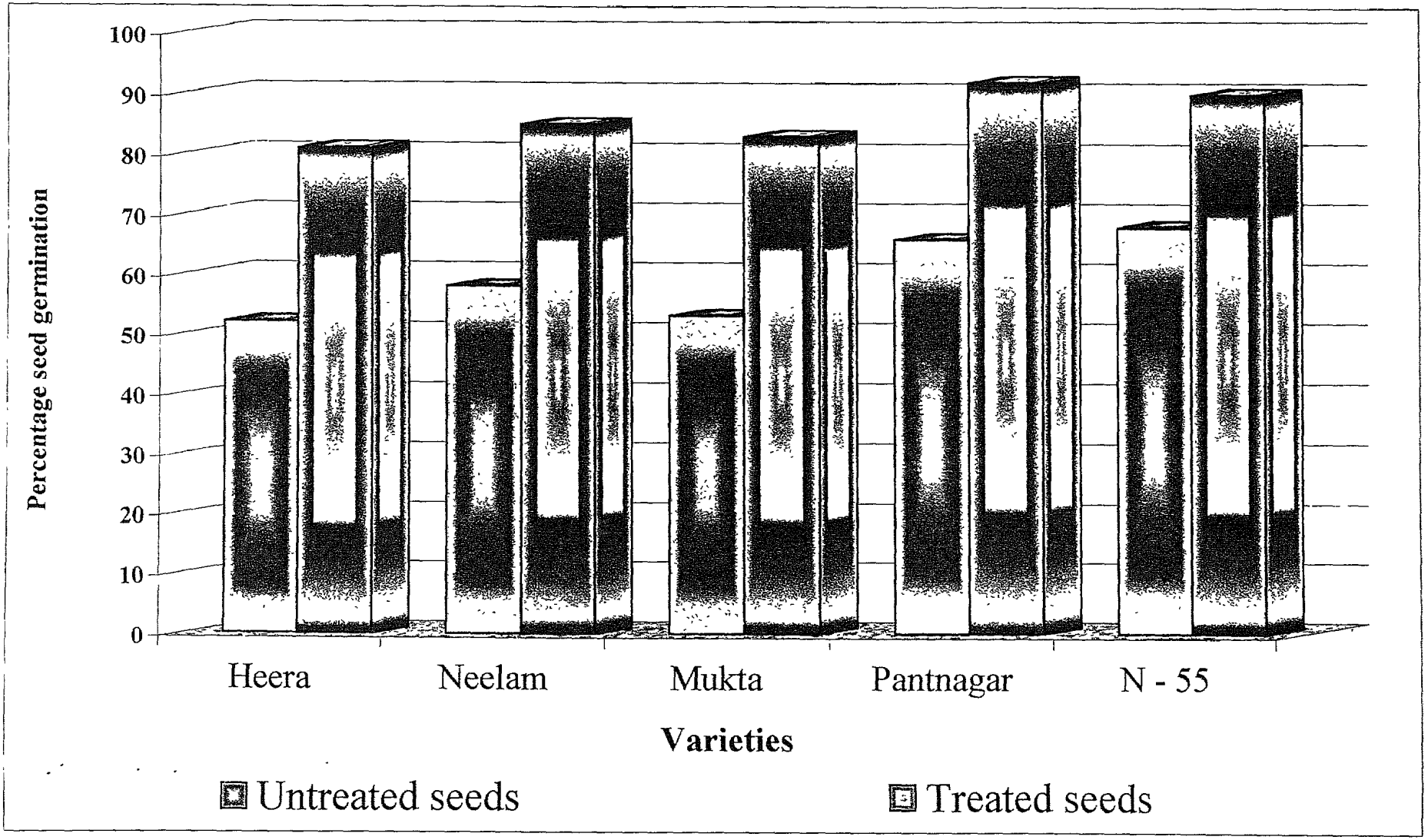
F = *Fusarium spp.*

G = *Cladosporium spp.*

H = *Trichoderma spp.*

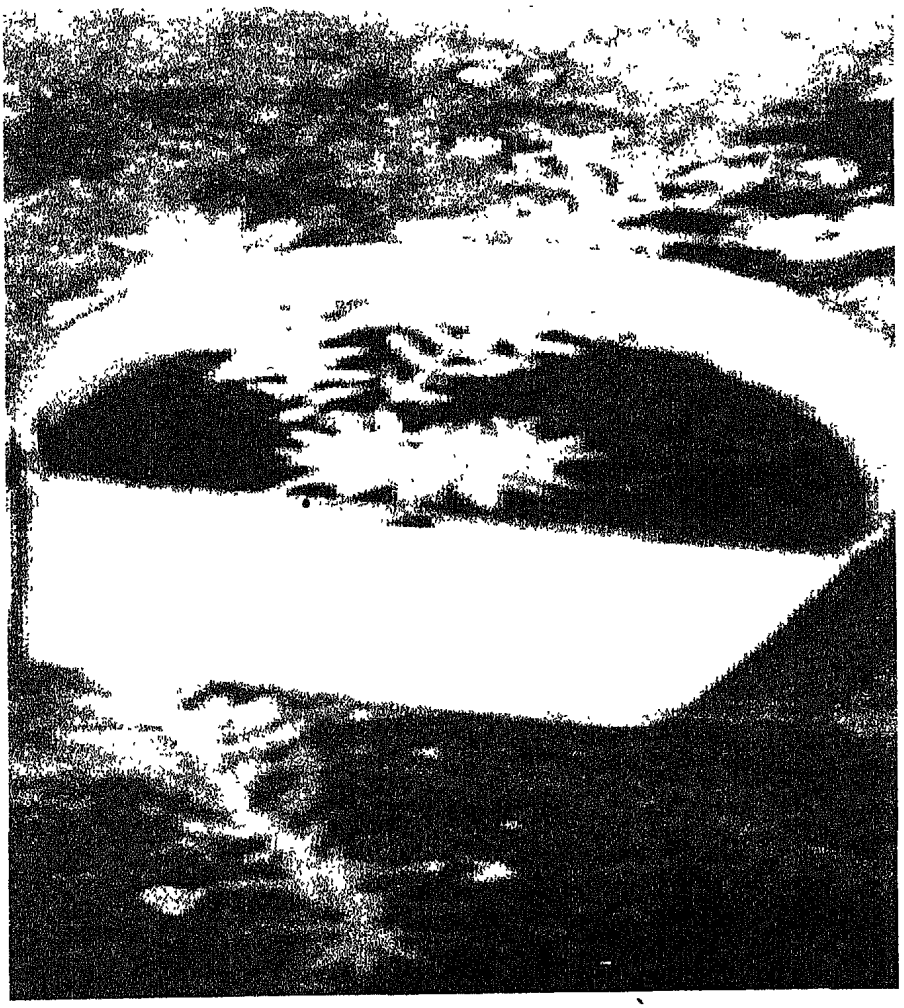
I = *Mucor spp.*

Percentage seed germination from some varietal samples of Linseed 'Flax' (*Linum usitatissimum* L.)



CHAPTER – 4

PATHOLOGICAL STUDIES



PATHOLOGICAL STUDIES

Seed-borne-diseases are quite common in oil seed crops. The pathogen may be adhering to the seed surface or they may be mixed with the seed lot. Since seeds are good substrate for fungi, they build up their inoculum potential on the seeds during germination and even kill the seedlings. Establishment of seed infection is more complicated. Many factors, particularly physiological conditions both of the pathogen and the host in conjunction with weather conditions are responsible for it. A few seed borne pathogens of oil crops have been thoroughly investigated with regard to the precise cause of transmission, including establishment of infection and development of the disease in the subsequent crops.

Fungi which were found to cause various rots (seed-rot, root-rot, stem-rot, flower-rot and pod-rot), wilts and leaf spots and the symptoms produced by them are recorded in Table - 3.

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the isolates and planting them on sterilized moist blotter paper in Petridishes as well as planting them in pots filled with sterilized field soil. Suitable controls were also maintained. Method suggested by Kilpatrick *et al.* (1954) was used to confirm pathogenicity at seedling stage. Cleaned test tubes with 10 ml tap water were taken. A sheet of Whatman No. 2 filter paper was placed

over the end of a wooden plug (which can easily go inside the test tubes). The sides of the filter paper were pressed down and then rolled around the wooden plug. The rolled paper was then pushed down into the test tube, leaving the plant from out of the water. Tubes were plugged with cotton and sterilized. Various isolates were grown on P.D.A. Fungal discs of 5 mm were cut with the help of a cork borer. Such discs were placed at the centre of the filter paper platform. Earlier surface sterilized seeds of desired hosts were germinated and when their length was about 0.5 cm, they were aseptically transferred to the filter paper platform. Tubes were labelled and placed on the tube stand for 10 days. Six replicates were taken in each case.

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures were sprayed on injured as well as uninjured flowers and leaves of their respective host plants with the help of hand automizer.

Pods on plants were inoculated by pinpricked injury and spraying the spore suspension. Humidity was maintained by covering it with a polythene bag with some sterilized water at the base. Plucked pods were similarly inoculated and kept under moist glass chambers. Suitable controls were maintained for each treatment. In each case similar condition such as amount of inoculum, water and same kind of soil etc. were provided.

The same species of *Fusarium,* viz. *Fusarium oxysporum* (Ellis and Everh), *Fusarium solani* (Schlecht) which commonly occur in

cultivated soil of Allahabad as well as isolated from seed of linseed crops were taken for pathological studies. These studies were carried out from the same stock cultures and hence the method used for isolating, sub-culturing, etc., were similar to those described earlier. For pathogenicity tests, linseed seedlings of same size and age (4 weeks old) were taken. Pathogenicity tests were carried out by the following methods:

1. The seedling root-rot both uninjured and injured (10 injuries by sterilized needle per root) were dipped inspite suspension (about 100 spores per lower field of compound microscope) of *Fusarium* species. The seedlings were then replanted in plastic pots. Controls were simultaneously maintained.

2. The seedlings were kept in culture tubes containing 20 days old culture filtrates of *Fusarium* species. In case of control, seedlings were kept in sterilized distilled water. Daily observations were made.

3. Re-isolations were always made in order to confirm the infection with particular *Fusarium* species. Ten seedlings per treatment were taken in each case. The results of both set of experiments are recorded in Table - 4.

The result from isolation studies showed that in the case of linseed crop, maximum association of *Fusarium spp.* was observed in the treated as well as untreated seeds. Other fungi were isolated from the untreated seeds.

The fungi isolated from known varieties of linseed crop seeds were similar to those isolated from composite samples of unknown varieties.

Pathological studies revealed that *Fusarium solani* caused seed-rot, pod-rot and seedling rot. *Fusarium oxysporum* caused wilt, seed-rot and seedling rot of linseed. *Fusarium moniliforme* caused wilt and seedling rot of linseed. *Fusarium acuminatum* caused rot and wilt of linseed were also able to cause wilt, root-rot and seed-rot respectively. In case of root-rot and wilt, infection appears to be seed-borne as the disease could also occur in infested soil.

Result from the above pathogenicity test clearly show that all the six species of *Fusarium* were capable of causing wilt of linseed and seedling. The main symptom of wilting was as if the seedlings have suffered from water shortage. The wilting was characterised by gradual withering, yellowing and drying of leaves. Later on it was followed by death of seedlings. It was observed that there was slightly higher percentage of infection increase of injured root seedling than those uninjured out of *Fusarium oxysporum* was more pathogenic to linseed seedlings than the other two species, as it caused a higher percentage of wilting in the seedling.

The plants may be attacked by the wilt causing pathogen at any stage of their growth. The disease causes both pre and post emergence losses. The freshly emerged seedling and mature plants may be attacked by the pathogen. The disease was first observed in the field in

second week of November 2000. The disease causes severe damage in November - December 2000. There is a variety of intensity of the disease in different seasons. Apparently the periodical variation in the intensity of disease indicate its close relationship with the environmental factors, such as moisture, temperature and age of plants.

In the field sown with T_1 variety on 28th October 2000, the observations were taken at the interval of 15 days with a view to study the frequency of the occurrence of the disease. The wilted plants were seen appearing in the middle of November and the observations were taken on 21st November, 5th December, 20th December, 5th January, 20th January, 5th February, 20th February, 7th March and 22nd March. 100 plants were taken at random and noted the percentage of disease.

Table - 5 and its graphic representation clearly shows that the lowest percentage of wilt occurred on 20th January 2001 and assumed most severe on 21st November 2001. The severity has no relation with age but the soil temperature plays an important role in the occurrence of the disease.

EXTENT OF DAMAGE

The fields were selected for taking observations to ascertain the approximate extent of damage by this wilt disease of linseed. One field was selected at Banda and one field at Allahabad. Ten rows in each field were selected and counted after germination was complete. Wilted plants were spotted out, uprooted and counted as they were observed.

The total number of healthy plants and wilted plants in a row were counted and the wilt percentage was calculated.

From the table 6 and 7, it is clearly evident that the severe damage of crops was recorded in the fields of Allahabad and less in those obtained from Banda.

Table - 3 Showing pathogenicity test of linseed 'Flax' (*Linum usitatissimum* L.)

Pathogen	Disease	Symptoms
Fusarium oxysporum	Wilt seedling rot	Leaf chlorosis followed by necrosis, brown disclouration of vascular tissues of roots and stem.
Alternaria state of *pleospora infectoria*	Leaf blight	Scattered pale brown spots on the leaf surface. Smaller spots coalesce to form bigger spots, drying of leaves from tips is usually observed.
Aspergillus flavus	Seed-rot	
Fusarium solani	Seed-rot, Root-rot, Seedling blight and seedling-rot	Reduction in root elongation, extensive browning of roots, finally results in rotting of seedlings.
Alternaria alternata	Leaf spot	Purple to brown coloured spots appear on the leave tissues, which turn necrotic, and concentric rings are formed.

Pathogen	Disease	Symptoms
Macrophomina phaseolina	Seed-rot, root-rot	Brown to black discolouration of basal portion of the seedlings. Yellowing of lower leaves, become conspicuous giving pale or sick appearance to the seedlings.
Fusarium equiseti	Root-rot	Root system totally lacking, plants are quite conspicuous with dry foliage.
F. acuminatum	Root-rot and Wilt	Root-lets are either not fully developed or destroyed due to rotting. Plants are easily detached at soil level when pulled out
Fusarium semitectum	Seedling-rot	Brown discolouration at the base of the radical, dark brown to black spots on the hypocotyl and cotyledons, decay or seedlings which get covered with profuse fungal growth.

Pathogen	Disease	Symptoms
Rhizopus stolonifer	Root-rot	Roots show browning and are poorly developed, lower leaves of infected plants gradually loose their green colour, turn pale followed by defoliation.
Fusarium moniliforme	Root-rot and Wilt	Root-lets are either not fully developed or destroyed due to rotting. Plants are easily detached at soil level when pulled out.
Fusarium spp.	Seed-rot, root-rot	The roots and particularly the collar portion turns yellowish brown, plants could be pulled out easily.

Table - 4 Pathogenicity test and percentage wilting of linseed (*Linum usitatissimum* L) at seedling stage

Sl. No.	Name of varieties	Organism	Percentage of seedling wilt		
			After 8 days		After 5 days
			Uninjured	Injured	Culture filtrate
1.	Hira	*Fusarium oxysporum*	45	55	65
2.	Neelam	*Fusarium solani*	40	50	60
3.	Mukta	*Fusarium oxysporum*	55	60	65
4.	Pant-nagar	*Fusarium solani*	50	55	60
5.	N - 33	*Fusarium spp.*	46	58	66
6.	N - 55	*Fusarium solani*	50	67	74
7.	R - 17	Fusarium solani	40	50	60
8.	Control	-	NIL	NIL	NIL

Pathogenicity test and percentage wilting of linseed (*Linum usitatissimum* L.) at seedling stage

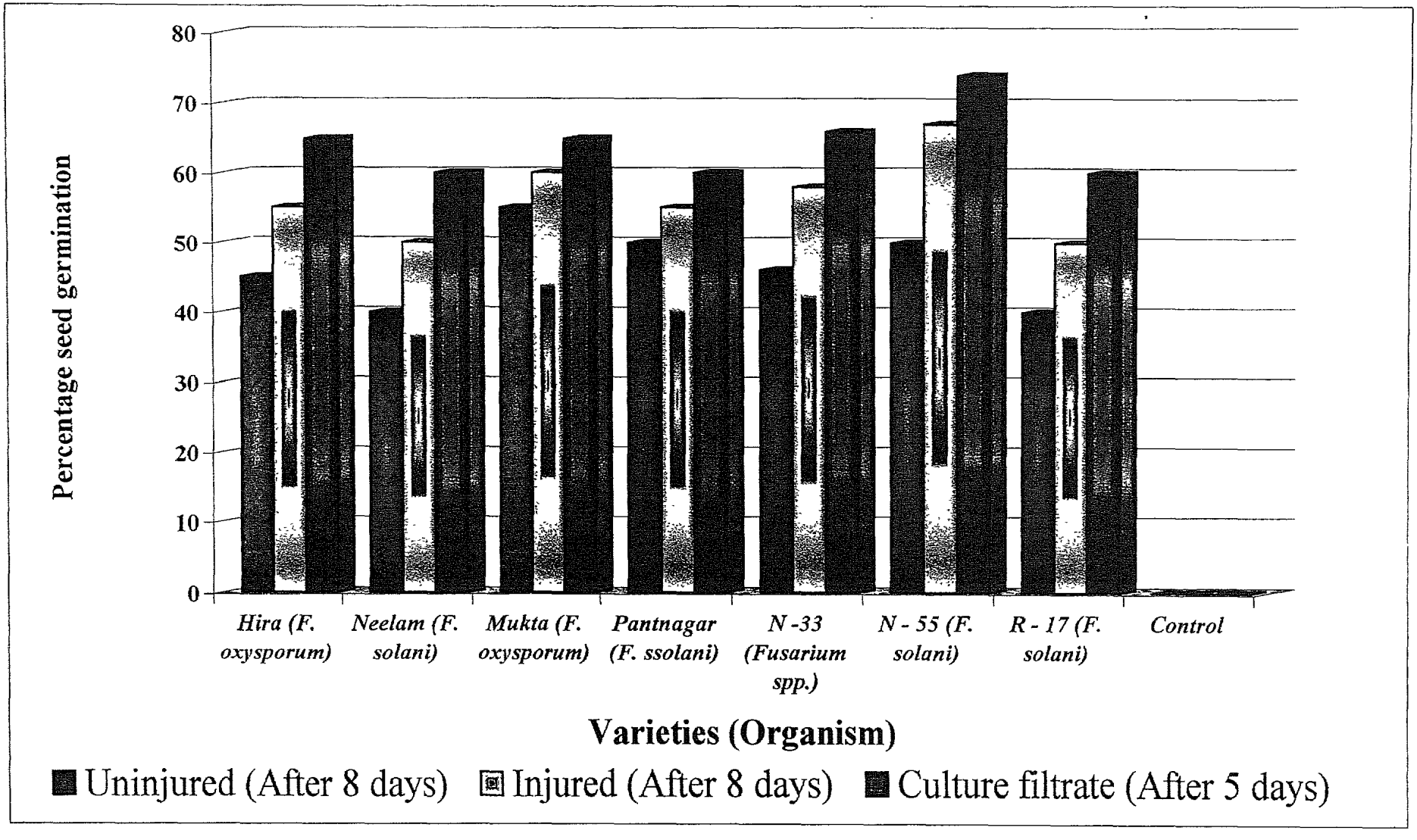


Table - 5 Showing percentage of disease occurring in different months (Year 2001)

Sl. No.	Date	Total No. of plants	Wilting percentage
1.	21st November	100	20
2.	5th December	100	19
3.	20th December	100	15
4.	5th January	100	7
5.	20th January	100	4
6.	5th February	100	8
7.	20th February	100	10
8.	7th March	100	12
9.	22nd March	100	9

Percentage of disease occurring in different months in Linseed 'Flax' (*Linum usitatissimum* L.)

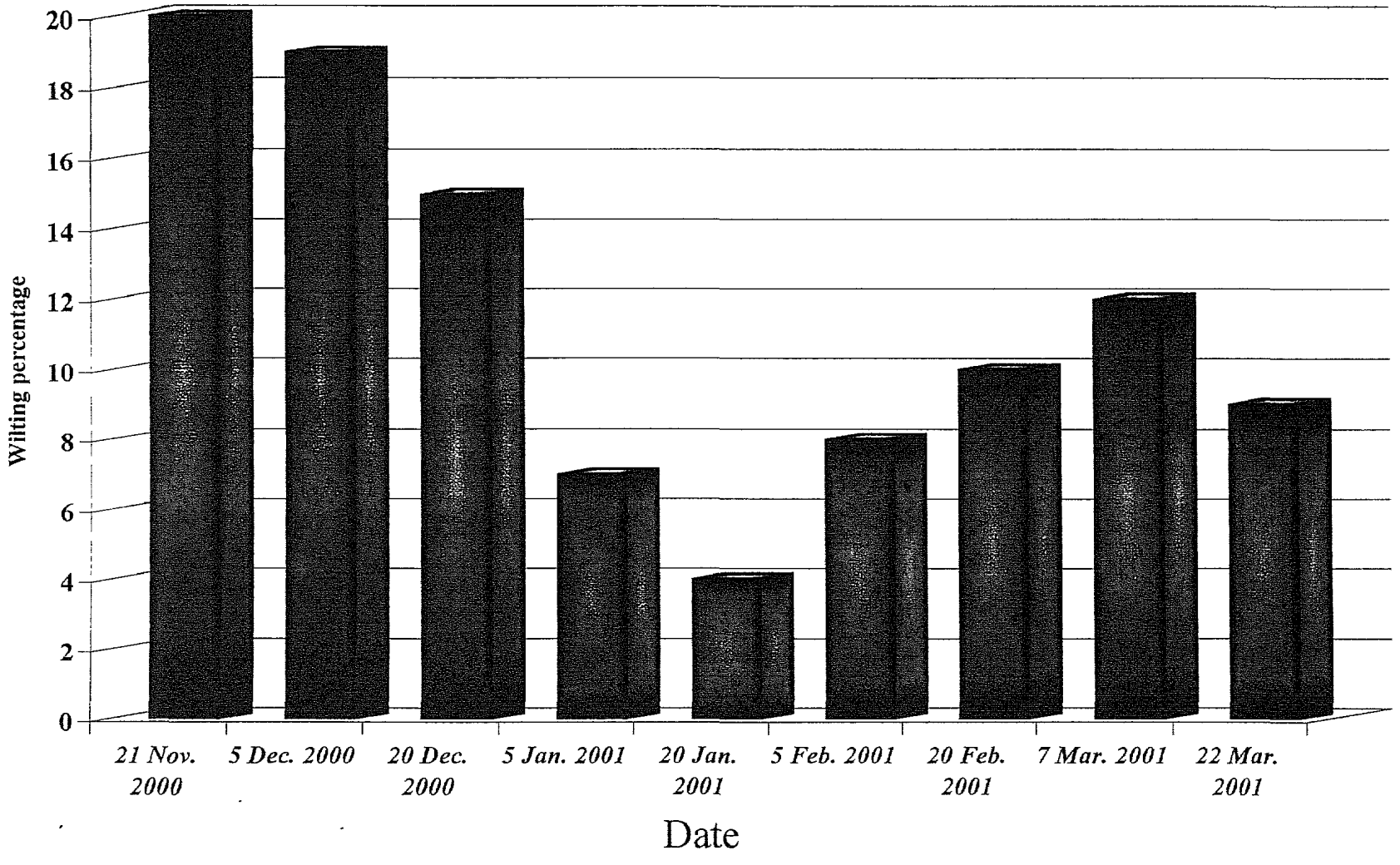


Table - 6 Showing percentage wilting of linseed collected from Banda

Sl. No.	No. of plants counted	No. of wilted plants	Percentage of disease
1.	580	46	7.9
2.	830	34	4.0
3.	797	20	2.5
4.	893	63	7.0
5.	509	53	10.9
6.	910	73	8.0
7.	353	29	8.2
8.	430	51	11.8
9.	690	70	1.1

Table - 7 Showing percentage wilting of linseed collected from Allahabad

Sl. No.	No. of plants counted	No. of wilted plant	Percentage of disease
1.	850	73	8.1
2.	760	75	9.3
3.	440	55	12.1
4.	460	40	8.1
5.	493	31	6.1
6.	1090	60	5.5
7.	891	40	4.4
8.	740	84	11.1
9.	650	55	8.6

CHAPTER – 5

PHYSIOLOGICAL AND BIOLOGICAL STUDIES

PHYSIOLOGICAL AND ECOLOGICAL STUDIES

INFLUENCE OF DIFFERENT TEMPERATURES

Temperature is one of the most important environmental factors, which play a significant role in governing various metabolic activities of fungi. According to Ten Hauntan (1960), "As weather is a complicated phenomenon most plant pathologists investigate it under laboratory conditions, e.g. the influence of temperature and humidity on the disease syndrome and on the pathogen, while keeping other environmental conditions constant. Afterwards they translate these findings into actual field conditions. Thus, many important results have been obtained, which have lead to a better understanding of pathogenesis and the epidemiological spread of the disease under investigation."

The range of temperature, which favours mycelial growth, varies considerably, depending upon the organisms concerned. Moreover, in nature, under prevailing humidity conditions hot or cold air and soil in which a crop and its parasites are growing may be decisive in determining the presence and severity of disease. According to Deverall (1965). "Temperature affects all the fungal cellular activities and it also causes shift in metabolism." The range of temperature, which favours mycelial growth, varied considerably depending upon the organism concerned.

Wolf and Wolf (1947) have mentioned that only a few fungi were active at 42°C. Usually fungi do not grow above 40°C or below 0°C. However, certain fungi are reported to grow even below the freezing point. Bidault (1921) as well as Brook and Hansform (1923) reported that *Cladosporium herbarum* could grow slowly at a temperature as .low as -6°C and even feebly at 10°C. Similarly, Pehrson (1948) found that *Phacidium infestans* was capable of growing at -3°C and the mycelium remained still variable after 138 days at -21°C. On the contrary, certain fungi have been found to grow at higher temperature. La Touche (1948) observed the growth of a species of *chaetomium* on straw at 62°C. Its maximum development was recorded between 40 and 50°C. However, fungal mycelium is easily killed by elevated temperatures and many fungi die slowly when held in culture at a temperature just above the maximum for growth. Thus fungi are more tolerant to lower than to higher temperature since, the latter coagulates cell proteins (Panasenko, 1967).

According to Togashi (1949) temperature optima (one of the three cardinal points at which mycelial growth of a fungal colony is best) for most of the pathogenic fungi lies between 20°C and 30°C and about a half have optima between 26°C and 30°C. Hawker (1950) pointed out that the optimum temperature for growth of fungi is usually between 20 °C and 30°C and the cardinal points (minimum, optimum and maximum temperature) for vegetative growth of the fungi are usually near 0 - 5°C, 20 - 30°C and 30 - 35°C.

Tisdale (1917) found that minimum temperature for growth of *Fusarium lini* was 10°C and the optimum was 26 - 28°C. Studies made by Edson and Shapolov (1920) showed that the minimum, optimum and maximum temperatures for *F. coeruleum* and *F. trichothecioides* were 5°C, 25°C and 35°C, respectively.

Moore (1924) working with *F. coeruleum* failed to get any growth of the fungus at 5°C, the optimum being 20°C and 25°C. Massey (1926) observed that *F. oxysporum* on Gladioli could grow over a range of 5 - 35°C with optimum temperature at 27.50°C. Agarwal (1955) reported that *F. coeruleum* could not sporulate at 8°C but the sporulation was best at 20- 24°C and it decreased at higher temperatures.

Rose (1960) reported that *Fusarium culmorum, F. sambucinum* var. coeruleum and *F. oxysporum* grew best at 28°C. Bhargava (1962) obtained best growth and sporulation of *F. solani* at 25°C. Bhatnagar (1967) observed a considerable variation in the production of microspores and macrospores in two isolates of *F. solani* and reported that as the temperature increased the production of chlamydospores increased and at 34°C only the later were formed. According to Joffe and Palti (1972), in cultures, isolates of *F. solani* grew best at 24 – 32°C.

It would be evident from the above facts that it is necessary to have a thorough knowledge of temperature requirements of the pathogens concerned. This would also provide an idea about the environmental conditions, which would be most suitable for propagation and survival of the pathogens in nature. Hence, the

influence of different temperatures, i.e. 5, 10, 15, 20, 25, 30, 35 and 40 °C on growth and sporulation of *F. oxysporum, F. solani* and *F. equiseti* has been investigated. The results are summarised in table - 8.

It is evident from the Table - 8 that all the three organisms were capable of growing within a wide range of temperature. They exhibited maximum mycelial growth at 25°C, similar to *F. culmorum* (Ross, 1960), *F. solani* (Joeffe and Patti, 1972) and *Fusarium sp.* (Shukla, 2000).

F. oxysporum exhibited poor fruiting bodies at 10°C and 15°C. *F. solani* developed poor sporulation at 10°C and 15°C. However, Agarwal (1987) reported poor sporulation of *C. verruculosa* at 15°C. *P. lycopersici* produced poor sporulation at 15°C.

F. oxysporum and *F. solani* failed to sporulate at 5°C and 40°C while *F. equiseti* failed to produce fruiting bodies at 5, 10 and 40°C.

In all the cases where growth was observed the initial pH of the medium shifted towards neutral or alkaline side.

It is clear that all the three fungi under study grow best at 25°C and the sporulation was also excellent at this temperature. Attempts were made to carry out subsequent studies on different organisms at optimum temperature except where otherwise stated.

From the above-mentioned facts, it would be evident that before starting any physiological experiment, it is indispensable to have a thorough knowledge about the temperature requirements of the organisms concerned.

Moreover, this would also give an idea about the environmental conditions that would be most suitable for survival and propagation of a pathogen in nature. Therefore, it was considered necessary to determine the cardinal temperatures and especially the optimum temperature for growth and sporulation of the present isolates. The temperatures were taken at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C.

Sporulation as well as chlamydospore formation of the present organisms was predominantly influenced by the temperature variations. Sporulation varied from poor to fair good in all cases which failed to sporulate at 10°C chalmydospore formation was better in all the three species when the temperature was unfavourable, i.e. 10°C and 35°C.

INFLUENCE OF DIFFERENT HYDROGEN ION CONCENTRATIONS

Hydrogen ion concentration greatly influences the growth and reproduction of fungi. pH is directly related with the processes like production of pigments, vitamins, antibiotics and other metabolites. According to Lilly and Barnett (1951) "The chemical changes in media due to alteration of pH, whether imposed from the outside or caused by the fungus, affect metabolic processes. The pH of a culture medium

changes during the growth of a fungus, and these changes may affect the composition of the medium and thus the response of the fungus."

The hydrogen ion concentration affects permeability of protoplasmic membranes, uptake of minerals, entry of essential vitamins and organic acids into the cell, activities of enzyme system, synthesis and stability of proteins and other life processes. The pH of the medium is changed by the growth of the fungus, at the same time pH also change the growth pattern of the organism. A highly acidic or a highly alkaline media inhibits the growth of most of the fungi.

Fungi generally utilise a substrate in the form of solution, and only if the reaction of the solution is conducive to fungal growth and metabolism (Bilgrami and Verma, 1978). During their growth they modify the pH of the medium and this may have its effect on their subsequent growth. Such an effect is caused due to uptake or release of anions or cations from or to the medium.

Cochrane (1958) as well as Tandon (1961) pointed out that most plant pathogens, with a few exceptions, grow best on media with an initial pH of 5.0 - 6.5. According to Lilly and Barnett (1951), "An initial pH of 5.0 to 6.0 is satisfactory (not necessarily optimum) for the majority of the fungi." It has also been observed that the pH of the most of the host plants generally ranges between 5.0 - 6.5. This may favour the establishment of the parasite on the host.

Panasenko (1967) explained that the fungi can develop at relatively wide range of pH but their enzymatic activity, which

regulates their metabolism, takes place within a much narrower range. Usually this range is between pH 5.0 to 6.0 but it is not an optimum for all of them. It has been observed that fungi show best growth in a particular range of pH. In general, the optimum for vegetative growth is sometimes wider than the optima for sporulation. In fact, the optima for spore germination are comparatively narrower as compared to that for vegetative growth.

Growth of fungi usually takes place over a fairly wide range of hydrogen ion concentration. Hawker (1950) reported that growth of *Fusarium fructigenum* or Richard's solution (4.6 pH) gradually raised the pH until the reaction turned strongly alkaline. Srinivaspai (1953) reported that *Fusarium vesinfectum* and *Fusarium moniliforme* could grow at considerably lower pH of 2.1. Bhargava (1962) found that optimum range for growth was 4.5 to 6.5 for *Fusarium solani.*

In the light of the above-mentioned facts, it was decided to study the effect of different pH values on growth and reproduction of the three organisms included in the present study. The basal medium was adjusted at pH 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0 and 10.0.

The average dry weight, sporulation and the final pH of the media are recorded in table - 9.

It is clear from the table - 9 that the growth of *F. oxysporum* and *F. solani* was maximum at pH 6.0. *F. oxysporum* isolated from "Til" (Shukla, 1982) and "Guava" (Mishra, 1992) showed maximum growth of *F. oxysporum. F. oxysporum* attained good growth between pH 4.0 -

7.5 and pH 4.0 - 7.0 respectively. Thus it slightly differed from the present isolate. The growth was moderate at pH 4.0 and 7.5 and poor at pH 2.0 - 3.0 and pH 8.0 - 10.0. Mishra (1992) reported moderate growth at pH 7.5 and Shukla (1982) reported at pH 3.0, 3.5 and 8.0. Thus, their organisms differ from the present fungus. *F. solani* developed good growth at pH 4.5 - 7.0 and poor growth at pH 2.0 - 4.0 and pH 7.5 - 10.0. *F. equiseti* attained good growth at pH 4.5 - 7.0, moderate at pH 4.0 and poor at pH 2.0 - 3.0 and pH 7.5 - 10.0.

F. oxysporum sporulated excellently between pH 5.5 - 6.5 similar to *F. oxysporum* (Shukla, 1982). The sporulation of *F. oxysporum* was good at pH 5.0 and 7.0 and fair at pH 4.5, 7.5 and 8.0 and poor at pH 4.0 and 9.0. Mishra (1992) reported poor sporulation of *F. oxysporum* at pH 9.0. *F. oxysporum* failed to sporulate at pH 2.0, 3.0 and 10.0. *F. solani* developed excellent sporulation at pH 5.0 - 6.0 similar to *F. speci* (Tandon and Srivastava, 1963). Good sporulation of *F. solani* was secured at pH 4.5, 6.5 and 7.0 and fair at pH 4.0 and 7.5 and poor at pH 3.0 and 8.0, but it failed to sporulate at pH 2.0, 9.0 and 10.0. *F. equiseti* sporulated excellently between pH 5.5 - 6.5. The sporulation of *F. equiseti* was good at pH 5.0 and 7.0 and fair at pH 4.5 and 7.5 and poor at pH 4.0 and 8.0 but if failed to sporulate at pH 2.0, 3.0, 9.0 and 10.0.

In the present investigation, the initial pH of the medium drifted towards neutral to alkaline side. In highly alkaline media this was towards lesser alkalinity and in highly acidic media the drift was towards less acidic side. According to Lilly and Barnett (1951), "These changes in pH are due to changes in the relative amounts of the acids

and bases formed or withdrawn and to the ionization constants of these compounds."

The above results indicate that the present organisms could grow within a wide range of pH, but the acidic medium appeared comparatively more favourable for their growth and sporulation.

Thus, it is evident from the above results that the best growth and excellent sporulation of *F. oxysporum* and *F. equiseti* were at pH 6.0, while *F. solani* did show at pH 5.5. Hence in all subsequent studies the initial pH of the medium was adjusted to these pH levels.

EFFECT OF CARBON : NITROGEN RATIOS

According to Cochrane (1958) the possible interaction between carbon and nitrogen sources should be considered first, because in some cases it may be necessary to study all putative carbon sources in media with different nitrogen sources. Asthana and Hawker (1936) reported that dilution of the entire medium or of glucose only, accelerated the development of perithecia in *Melanospora destruens*. Reduction of nitrogen level did not influence perithecial development. Baker (1931) however, found that low concentration of carbon favoured the zygospore production in *Sporodinia grandis* when nitrogen content was increased. Talley and Balnk (1941) observed that if carbon sources were not a limiting factor, the rate of growth was controlled by the

concentration of nitrogen, but if carbon was a limiting factor, there was no significant increase in the growth of *Phymatotrichum omnivorum* by increasing the amount of nitrogen. Buston and Basu (1948) and Mathur *et al.* (1950) found that the growth and sporulation of *Chaetomium globosum* and *Colletotrichum lindemuthianum* respectively increased with increasing amount of carbon upto a limit after which the growth increased while sporulation decreased.

The carbon and nitrogen ratio may also influence the type and form of spores produced. Nitimargi (1935) observed that the spores of *Phomopsis* and *Diaporthe* vary from small and oval to large and curved or elongated and sickle-shaped, as the ratio of glucose to asparagine is increased. Cultural characters are also reported to be influenced by increasing supply of carbon. Pine (1958) also observed some changes in characters of four isolates of *Phomopsis vilicola* when subjected to increasing carbon supply.

Sometimes a low C : N ratio may be harmful because it has been observed that in the presence of low carbon supply, a decrease in the population of root pathogens under a high C : N ratio in the soil has been demonstrated in several instances. Snyder *et al.* (1959) controlled the bean root rot caused by *F. solani, F. phaseolina* with soil amendments having high C : N ratio. Similarly Dhingra and Sinclair (1976) also deserved a decline in the *Sclerotium* population of *Macrophomina phaseolina* at high C : N ratio. Soils were amended with glucose and sodium nitrate in different C : N ratios. Amendments to provide C : N ratios of 10, 20, 40 and 80 were based on 1 percent glucose

in air dried soil (w/w) and sodium nitrate. The soil samples were incubated for 4 weeks. Results are summarised in the Table - 10.

The population of all the three species of *Fusarium* declined in the soil samples amended with glucose and sodium nitrate mixture. It was also observed that as the C : N ratio was increased, the number of colonies declined more rapidly in the soils. The population of *F. equiseti* and *F. oxysporum* for all C : N ratios decreased in comparison with control upto the end of 4 weeks. The population of *F. solani* increased during the first week then declined upto the end of incubation period.

EFFECT OF SOME FUNGICIDES IN SOIL

Workers including Valaskova (1962), Sen Gupta and Roy (1971) and Ilyas *et al.* (1975) have tested a number of fungicides for soil disinfection. In view of this fact, a number of fungicides were tried to check the population of *Fusarium* species in soil.

In a preliminary test, the efficiency of a number of fungicides viz., Aureofungin (a heptaene antibiotic), Benlate (Methyl) 1-(butylearnamoyl) 2- benzimidazolecarbamate), Brassicol (Pentachloronitrobenzene) Cercobin (Benzene thiophanate), Difolatan (Cis-N-1(1,1,2,2-tetrachloroethyl) thio-4-cyclo-hexene-4, 2-dicarboximidie), *Ferbam* (ferric dimethyldithio-carbamate), *Plantvax* (DCMOD), 2, 3 dihydro-5 carboxanilido-6-methyl-1, oxathiin-4, 4 dioxide), thiram (tetramethyl thiuram disulphide or bis (dimethyl - thio

- carbomoyl) disulphide) and Vitavax (DMOC, 5, 6-dihydro-2methul - 1, 4-oxathin-3-carboxanilide against three species of *Fusarium* was tried in vitro. The fungicides were added to the basal medium at different concentrations (50 - 1000 ppm) and flasks was steam sterilized for 3 successive days for 30 minutes. Results are recorded in Table - 11.

The results show that Benlate at 50 ppm, Cercobin at 500 ppm, Difolatan 100 ppm, Plantvax at 500 ppm and Vitavax at 1000 ppm were found to be inhibitory to the growth of present organisms, whereas rest of the fungicides including the antibiotic. Aureofungin failed to inhibit the growth of present *Fusarium* species even at the concentration of 1000 ppm (maximum concentration taken). The concentrations of fungicides, which inhibited the growth of the organisms, were then employed to check the population of *Fusarium* species in soil. In addition to this, Tecto '40' (Thiabendazole (42.8 percent), 2-(4-Thiazolyl) benzimidazole was also used.

All the fungicides were amended in the soil at a concentration, which was found to be inhibitory to the growth of the organisms in culture media. Tecto '40' was amended to the soil at a concentration of 500 ppm. The fungal inoculum was incorporated in the soil after 24 hours of addition of the fungicide. The results are summarised in the Table - 12.

Difolatan was found to have a marked retarding effect on the survival of all the three species of *Fusarium*. At a concentration of 100 ppm, the fungal survival was almost eliminated. The incidence of

survival of *F. equiseti* and *F. solani* was slightly increased in case of Vitavax and Cercobin respectively as compared to other fungicides. Tecto '40' was quantitatively less effective than Benlate in reducing the population of present species of *Fusarium*. Similar results were also obtained by Ilyas *et al.* (1976) for *Macrophomina phaseolina*.

ECOLOGICAL STUDIES

Some soil-borne wilt diseases affected by the soil composition have been reported by many workers (Young, 1928; Bolley and Manna, 1932; Walker and Snyder, 1934). Similar results were observed by Vasudeva and Srinivasan (1952) for *Fusarium* wilt or lentil and by Shukla (1972, 1975) for wilt of guar and *Fusarium* wilt of arhar. The wilt was maximum. In the present study almost similar results have been obtained. More the proportion of sand in the soil, more is the percentage of wilting. This is perhaps due to the sufficient pore space provided by sand particles, which help in free traversing and branching of hyphae that penetrate the tissues of rootlets.

The present study deals mainly with the soils of Allahabad Agricultural Institute, Naini - Allahabad and Government Agricultural Farm, Allahabad.

The soils of these places were found to be generally sandy-loam (Genetic alluvial). Quarterly collection of soil samples from different fields was made for three years (January 1999 to October 2001).

Methods of isolation were similar to those described earlier. Meteorological data for the year was obtained from the Air Force Meteorological Station, Bamrauli. The pH values, moisture contents, percentage of nitrogen, organic carbon and organic matters of the soil were also determined. The pH was determined with the help of Lamotte's soil testing outfit. Percentage of nitrogen and organic carbon were estimated by Kjeldahl's and Walkley and Black's rapid titration methods, respectively. The number of *Fusarium* colonies isolated was based on five replicates per sample. The results are summarised in Table - 13.

In general such variations in the *Fusarium* population were observed round the year but the trend of *Fusarium* population in the soils of three different areas was similar. In all the cases maximum number of colonies were isolated in October followed by January and April. Minimum number of colonies were isolated during July, thus number of colonies differ with the seasons and locations.

Temperature has markedly affected the population of *Fusarium* species in the present soils. The results clearly showed that when the temperature was high, i.e. during April, the number of colonies isolated were less, being minimum in July might also be due to heavy rains which resulted in poor aeration of the soils.

Maximum number of colonies were isolated in the month of October clearly showed that the condition were optimum. The environmental conditions as well as other conditions in general, such as

the percentage of organic carbon and nitrogen were comparatively better than in other months.

Moisture, which is important for the growth of soil fungi, has a pronounced effect on their distribution. In water logged fields anaerobic conditions affect them adversely. Saxena (1955) has reported that under such conditions only those forms thrive which are adapted to aquatic conditions such as species of *Allomycee* etc., members of *saprolegniaceae* and *phythieceae*.

During summer due to drought (less of moisture) and high temperatures, the colonies were found in less numbers. The minimum relative humidity at which fungi are able to grow is correlated with the concentration of the cell sap and consequently the suction pressure exerted by the hyphae.

It is the general view of many workers that fungi grow luxuriantly in acid soils but bacteria predominate in alkaline soils, while Jonsen (1931) and others proved that fungi are also abundantly found in alkaline soils and play a dominant role in the microbial activity of such soils (Waksman, 1927). In the present study, the pH of soils generally ranged between 6.8 and 8.0 being neither very acidic nor alkaline.

Table – 8 Average dry weight (mg), sporulation and change in pH when *F. oxysporum, F. solani* and *F. equiseti* were grown at different temperatures

Treat-ment No.	Temp-erature in °C	*F. oxysporum*			*F. solani*			*F. equiseti*		
		Dry wt.	Sporulation	Final pH	Dry wt.	Sporulation	Final pH	Dry wt.	Sporulation	Final pH
1	5°C	0.0	N	5.5	0.0	N	5.5	0.0	N	5.5
2	10°C	22.4	P	5.8	29.5	P	5.5	22.0	N	5.5
3	15°C	56.4	F	6.4	57.6	P	7.0	36.5	P	6.0
4	20°C	87.7	G	7.0	87.6	G	6.5	102.7	G	6.5
5	25°C	126.5	E	7.2	117.2	E	6.5	120.6	E	7.0
6	30°C	99.8	G	7.0	102.9	E	7.2	96.2	E	7.0
7	35°C	69.7	P	6.5	74.3	F	7.2	56.7	G	6.5
8	40°C	10.7	N	6.0	0.0	N	5.5	10.6	N	6.0
	G. M.	59.2			58.6			55.7		

Summary of the dry weight results and conclusions at 5% level of p:

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non-significant	Non-significant	Non-significant
S. E.	1.0	1.2	1.1
C. D. at 5% level	± 4.2	± 4.3	± 3.7

Treatment Nos.

F. oxysporum 5 > 6 > 4 > 7 > 3 > 2 > 8 > 1

F. solani 5 > 6 > 4 > 7 > 3 > 2 > $\overline{1 \quad 8}$

F. equiseti 5 > 4 > 6 > 7 > 3 > 2 > 8 > 1

Change in pH when *F. oxysporum* , *F. solani* and *F. equiseti* were grown in different temperatures

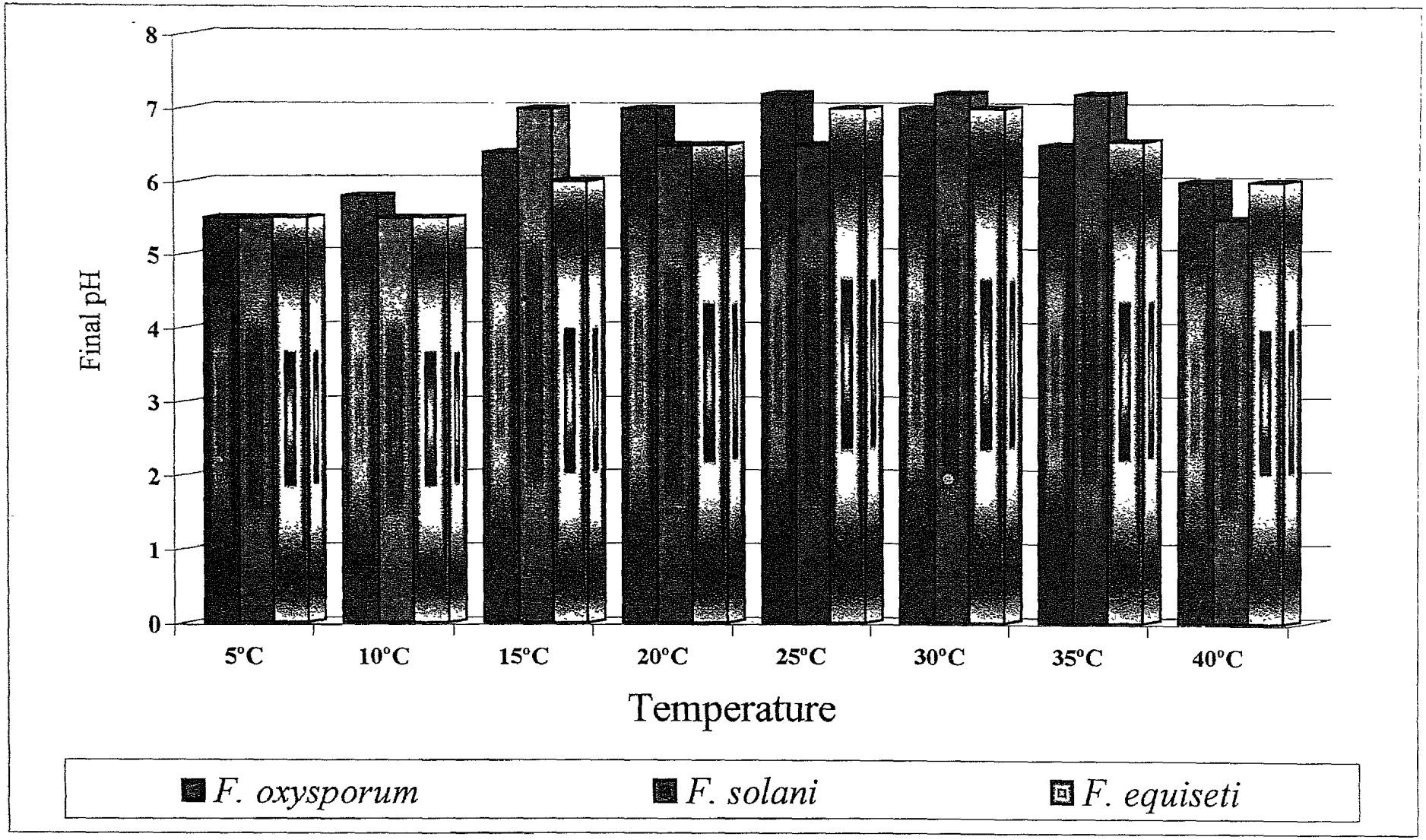


Table – 9 Average dry weight (mg), sporulation and change in pH caused by *F. oxysporum*, *F. solani* and *F. equiseti* at different pH values

Treatment No.	Initial pH	*F. oxysporum*			*F. solani*			*F. equiseti*		
		Dry wt.	Sporulation	Final pH	Dry wt.	Sporulation	Final pH	Dry wt.	Sporulation	Final pH
1	2.0	23.4	N	4.5	25.2	N	4.5	21.7	N	4.0
2	3.0	52.3	N	5.5	54.5	P	5.0	43.9	N	5.5
3	4.0	74.7	P	5.5	68.0	F	5.5	61.9	P	5.5
4	4.5	82.8	F	6.0	95.5	G	6.0	71.1	F	6.2
5	5.0	96.4	G	7.1	115.8	E	6.5	93.6	G	6.5
6	5.5	116.1	E	7.4	120.0	E	6.5	104.2	E	7.2
7	6.0	126.4	E	7.8	117.0	E	7.2	116.1	E	7.0
8	6.5	117.4	E	7.8	99.1	G	7.2	96.1	E	7.5
9	7.0	82.6	G	8.2	82.5	G	7.5	77.8	G	7.5
10	7.5	69.9	F	8.4	68.4	F	8.2	56.8	F	8.0
11	8.0	57.8	F	8.4	56.2	P	8.2	42.3	P	8.2
12	9.0	34.2	P	8.6	37.6	N	8.5	35.0	N	8.5
13	10.0	23.7	N	8.6	22.2	N	8.5	21.1	N	8.5
G. M.		73.7			74.0			64.1		

Summary of the dry weight results and conclusions at 5% level of p:

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non-significant	Non-significant	Non-significant
S. E.	1.5	1.1	1.2
C. D. at 5% level	± 4.7	± 3.4	± 3.7

Treatment Nos.

F. oxysporum 7 > 8 6 > 5 > 4 9 >3 > 10 > 11 > 2 > 12 > 13 1

F. solani 6 7 5 > 8 > 4 > 9 > 10 3 > 11 > 2 > 12 > 1 13

F. equiseti 7 > 6 >8 > 5 > 9 > 4 > 3 > 10 > 2 > 11 > 12 > 1 > 13

Change in final pH caused by *F. oxysporum*, *F. solani* and *F. equiseti* at different pH values

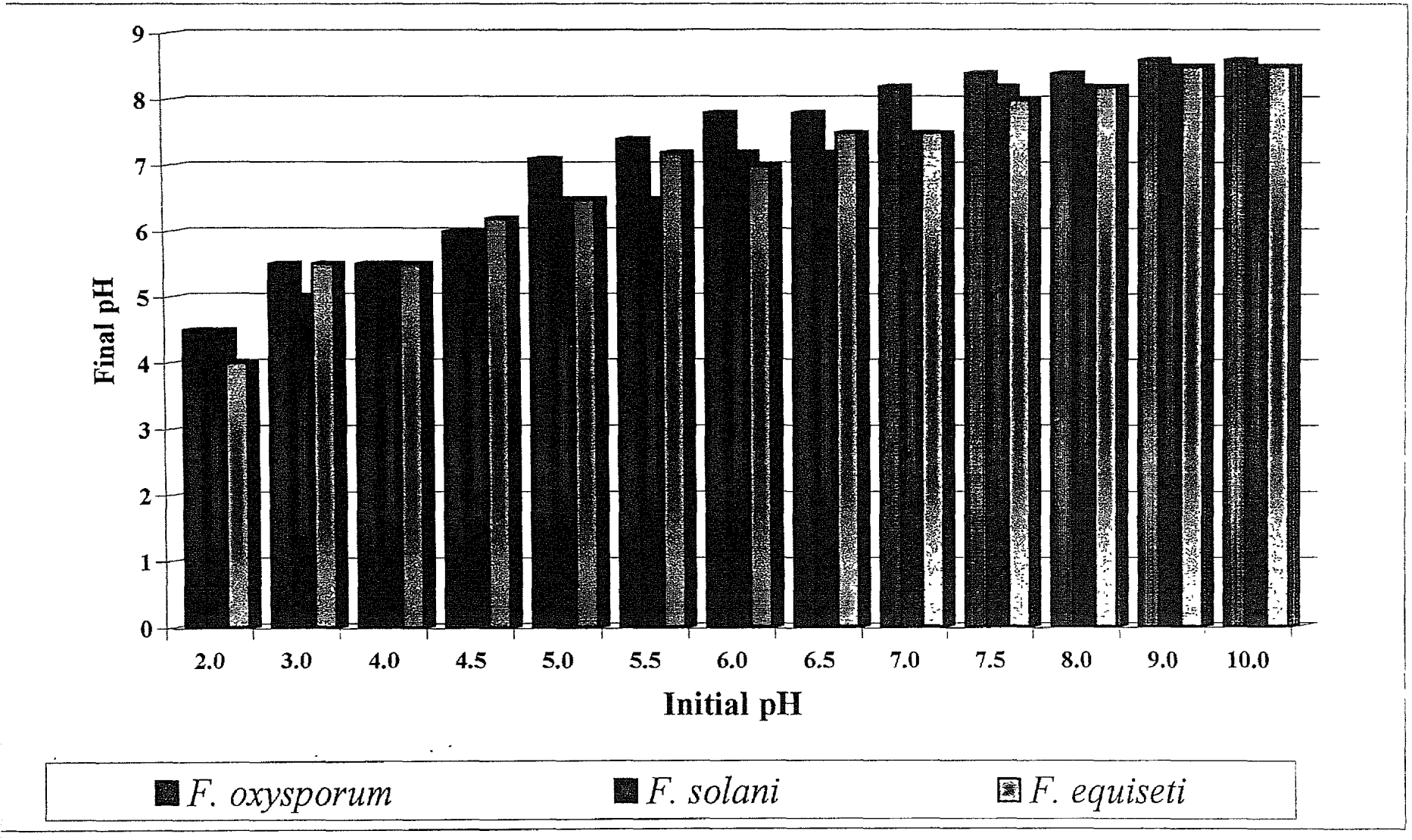


Table – 10 Effect of C : N ratios on *Fusarium* species population in soil

Fusarium Species	Treatment C:N ratio *	Number of *Fusarium* colonies after		
		1st week	2nd week	4th week
F. Solani	10	85	44	20
	20	59	38	21
	40	42	27	18
	80	19	19	2
	Control	63	62	66
F. oxysporum	10	14	9	3
	20	6	4	4
	40	4	0	0
	80	1	0	0
	Control	75	75	90
F. equiseti	10	35	20	8
	20	27	10	10
	40	9	16	11
	80	1	0	0
	Control	72	52	75

* Based on 1 percent Glucose in air dried soil (w/w) and Sodium nitrate

Table - 11 Effect of some fungicides on growth (Dry weight) and sporulation of *Fusarium* species

Fungicide	Concen-tration (ppm)	*F. solani*		*F. oxysporum*		*F. equiseti*	
		Dry wt. (mg)	Sporu-lation	Dry wt. (mg)	Sporu-lation	Dry wt. (mg)	Sporu-lation
Aureofungin	50	59.2	Good	57.2	Poor	59.8	Poor
	100	48.8	Poor	33.4	-	49.0	-
	500	29.2	Poor	15.8	-	26.2	-
	1000	11.2	-	7.4	-	9.2	-
Benlate	50	-	-	-	-	-	-
Brassical	50	67.2	Excellent	63.8	Good	89.7	Excellent
	100	64.8	Good	57.3	Fair	77.8	Good
	500	55.2	Fair	53.8	Fair	62.4	Poor
	1000	41.8	Fair	38.6	Poor	54.6	Fair
Cercobin	50	20.4	-	-	-	41.4	Poor
	100	-	-	-	-	19.9	-
	500	-	-	-	-	-	--
Difolatan	50	31.6	-	9.4	-	37.8	Poor
	100	-	-	-	-	49.0	-
Ferbam	50	59.2	Good	57.6	Poor	75.8	Fair
	100	53.8	Fair	53.4	Poor	68.0	Fair
	500	46.6	Poor	37.0	Poor	62.8	Poor
	1000	35.8	-	29.8	-	41.6	-
Plantvax	50	61.8	Good	52.8	Fair	75.0	Good
	100	59.2	Fair	47.0	Poor	55.8	Poor
	500	-	-	-	-	-	-
Thiram	50	63.0	Excellent	53.0	Excellent	67.8	Excellent
	100	59.8	Good	41.0	Fair	65.0	Good
	500	47.0	Poor	24.0	Fair	58.0	Fair
	1000	42.8	-	12.2	Poor	38.2	Poor
Vitavax	50	61.8	Good	56.0	Fair	62.8	Fair
	100	57.6	Good	43.2	Poor	55.0	Poor
	500	45.8	Poor	25.8	-	42.0	Poor
	1000	-	-	-	-	-	-
Control	-	76.0	Excellent	57.8	Excellent	61.4	Excellent

Table - 12 Effect of fungicide amendment in soil on *Fusarium* species population

Sl. No.	Fungicide	Concentration (ppm)	Number of colonies per 50 mg of soil		
			F. solani	*F. oxysporum*	*F. equiseti*
1.	Benlate	50	16	1	8
2.	Cercobin	500	42	4	18
3.	Difolatan	100	0	0	2
4.	Plantvax	500	17	8	12
5.	Tecto '40' (T.B.Z. 42.28%)	500	19	4	9
6.	Vitavax	1000	15	1	38
7.	Control	-	59	70	72

Table - 13 Distribution of *Fusarium* colonies in soils having *Linum usitatissimum* L. from three fields

Month	Field	Soil pH	Moisture	N_2	Organic Carbon	Organic matter	Atmospheric Temperature		Relative Humidity		Total Rainfall	*Fusarium* colonies per 50 mg soil
							Min.	Max.	Min.	Max.		
			%	%	%	%	°C	C	%	%	(mm)	
1999 JAN.	A	7.7	12.30	1.46	0.58	0.96	5.3	26.6	31	70	11.7	25
	B	8.0	14.48	1.14	0.54	0.94						33
	C	7.6	11.78	1.37	0.63	1.07						30
APR.	A	7.8	10.17	1.48	0.71	0.92	16.6	43.0	9	51	5.2	23
	B	7.7	19.27	1.18	0.54	0.91						30
	C	7.7	12.14	1.43	0.54	0.97						20
JULY	A	7.7	5.7	1.54	0.69	1.25	23.6	36.2	56	100	255.3	6
	B	7.8	12.28	1.12	0.62	1.10						14
	C	7.6	6.8	1.42	0.61	1.07						10
OCT.	A	7.7	3.7	1.42	0.71	1.28	15.0	36.4	30	88	12.6	37
	B	7.8	12.7	1.04	0.70	1.20						45
	C	7.7	6.9	1.37	0.68	1.10						32
2000 JAN.	A	7.6	1.9	1.34	0.69	1.21	3.6	26.2	29	72	0.1	28
	B	7.9	13.7	1.01	0.71	1.19						35
	C	7.7	7.8	1.18	0.74	1.30						34
APR.	A	7.5	7.7	1.22	0.82	1.41	13.6	43.2	6	19	Trace	26
	B	7.9	9.8	0.95	0.79	1.33						31
	C	7.5	9.6	1.07	0.94	1.70						22
JULY	A	8.0	22.4	1.10	0.91	1.50	23.8	38.7	54	95	342.6	8
	B	7.8	31.4	0.89	0.84	1.41						13
	C	7.8	26.4	1.07	0.94	1.71						12

Month	Field	Soil pH	Moisture	N_2	Organic Carbon	Organic matter	Atmospheric Temperature		Relative Humidity		Total Rainfall	*Fusarium* colonies per 50 mg soil
							Min.	Max.	Min.	Max.		
			%	%	%	%	°C	C	%	%	(mm)	
OCT.	A	7 5	18.9	1.34	1.01	1.60	14.8	35.6	25	79	2.3	41
	B	7.5	24.8	1 00	1.03	1.80						48
	C	7.4	25.2	1.18	1.19	2.10						36
2001 JAN.	A	6.9	17.9	1.48	1.59	2.68	2.3	31.2	32	100	0.0	26
	B	7.5	22.2	1.01	1.19	2.11						38
	C	7.3	23.4	1.30	1.79	2.89						31
APR.	A	6.9	16.8	1.20	1.00	1.56	10.0	45.2	7	42	9.6	20
	B	7.6	23.1	0.96	0.90	1.39						33
	C	7.1	25.0	1.35	0.89	1.38						25
JULY	A	6.8	12.8	1.41	0.69	1.21	24.0	35.0	61	97	345.6	5
	B	7.1	16.8	1.14	0.64	1.04						16
	C	7.0	20.0	1.40	0.66	1.07						12
OCT.	A	6.8	12.8	1.46	0.68	1.17	15.0	35.0	32	78	5.8	39
	B	7.2	15.8	1.14	0.61	1.03						49
	C	6.9	18.3	1.41	0.64	1.04						30

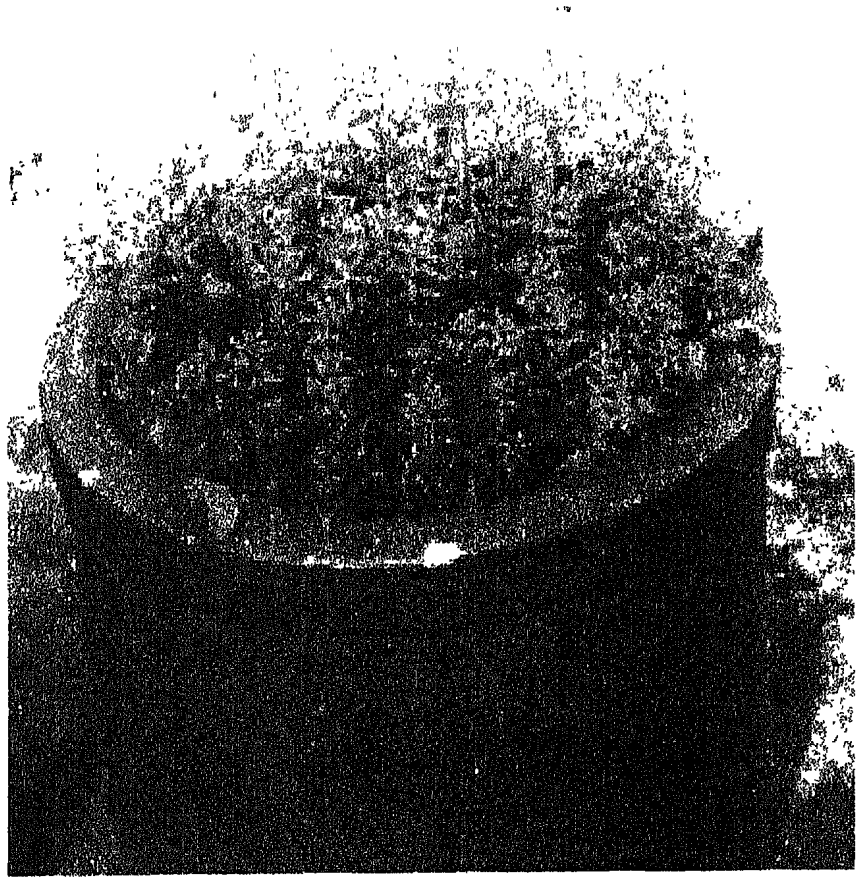
A : Agricultural Institute, Naini

B: Agriculture Farm, Allahabad University

C: Government Agriculture Farm, Allahabad

CHAPTER – 6

STORAGE STUDIES



STORAGE STUDIES

While harvesting of the seeds is seasonal, its consumption is continuous throughout the year. Therefore, crop should be stored for few months to at least one year, to ensure the continuous supply. It is said that without an ample storage of seeds, there can be no national treasure or no future for the nation.

Protection of seeds is exercised through a complex logistical system that moves seeds from producer to consumer. Quality is of concern throughout the whole system but control is normally practiced where storage facilities are available.

The storability is largely dependent upon the chemical changes, which take place in the seeds during storage, and is governed by temperature, humidity, aeration and microbial activities.

Storage fungi caused great problem during storage. Sick wheat mustiness, heating and caking and bin burning is all due to storage fungi and is products of poor storage conditions. However, to a farmer all this scientific research would be of little value unless he gets seeds which are genetically pure and possess all desired qualities namely, high germination percentage and vigour, high purity, sound health, etc. When the farmers do not get seeds possessing these qualities the yields they obtain may not be as expected. In other words, the seeds of hope may turn into seeds of frustration.

The scientific method of large scale or bulk storage of seeds is done either in silo elevators which may be made up of concrete or metal bins or in warehouse where the seeds are stored in bags which are kept 6 to 10 inches above the ground on wooden frames. These warehouses may have bolted steel walls with sloping roof or brick walls with sloping cement asbestos roof and are provided with arrangement for aeration.

Besides the above scientific methods, in India the seeds are also stored at farmers' or traders' levels in two types of storage structures. The one consists of underground pits lined from inside with straw ropes and husk and plastered with cow-dung to prevent seepage of water from surrounding soil. This is commonly known as 'Khattis', 'Matka', 'Kothi', 'Kuthla', 'Bukhari', 'Theka' and 'Bharola' with capacity of storing seeds from 2 to 100 quintals each, depending upon the size of shape. There are a considerable variety of structures, depending upon the local conditions and traditions.

Essentially adequate regulation of storage condition is to prevent infestation of insects and keep down humidity at a safe level.

In countries with a humid climate, such as Indonesia, the traditional system of storage, i.e. to put the grain on shelves in the kitchen above the oven is practised. In South Vietnam, grain is stored in cylindrical bamboo frames which are provided with a layer of palm leaves around the sides and on the bottom, moreover a layer of rice hulls is placed on the bottom and another layer on the top of grain butt. The container is placed in the kitchen, while in Ghana, maize intended

germinability, to avoid breakage in subsequent handling and to achieve low enough moisture content that will permit storage and shipment without spoilage, is becoming more and more important in national and international trade. In India, the drying of grains and seeds, so as to maintain high quality for food and feed, is accompanied by many difficulties. Here farmers dry seeds when it is raining or the weather is hot and humid. The aim in drying grain then is not to remove as much water as possible, but to remove as little as possible to meet a given grade or to make the grain safe for storage for a given length of time, at a given temperature.

$$\% \text{ Moisture content} = \frac{(W_1 - W_2)}{W} \times 100$$

Where W = Weight of sample

$W_1 - W_2$ = Difference between initial and final weight of seeds after drying

According to Papavizas and Christensen (1958), "Wheat with a moisture content upto 16 percent may be stored without obvious deterioration for a year at a temperature of 10°C or below and wheat with a moisture content upto 18 percent maybe stored safely for as long as 19 months at temperature of 5°C." Qasem and Christensen (1958) working with samples of corn stored in the laboratory at moisture contents of 16 and 18 percent and temperatures of 5°C, 10°C, 15°C, 20°C and 25°C stated, "Low temperature was as effective as low moisture content in preventing damage by the fungi tested."

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Table - 14 Percentage infestation of seeds of linseed (*Linum usitatissimum* L.) for different length of time

Duration	Percentage of infected seeds
Fresh seeds	15.63
Stored for 6 months	23.04
Stored for 1 year	19.78
Stored for 2 years	15.93
Stored for 2 ½ years	21.09
Stored for 3 years	12.02

Percentage infestation of seeds of Linseed (*Linum usittatissimum* L.) for different length of time

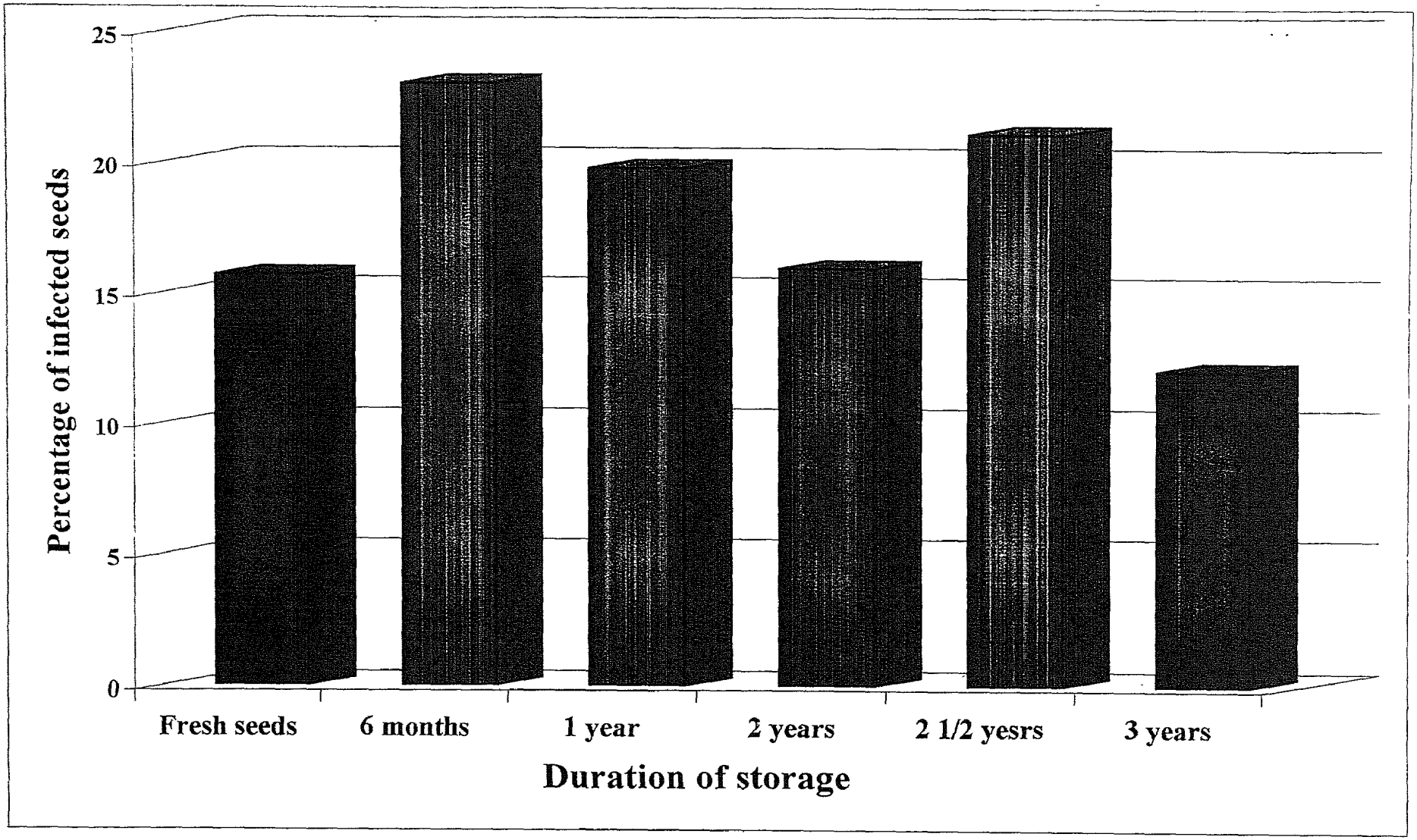


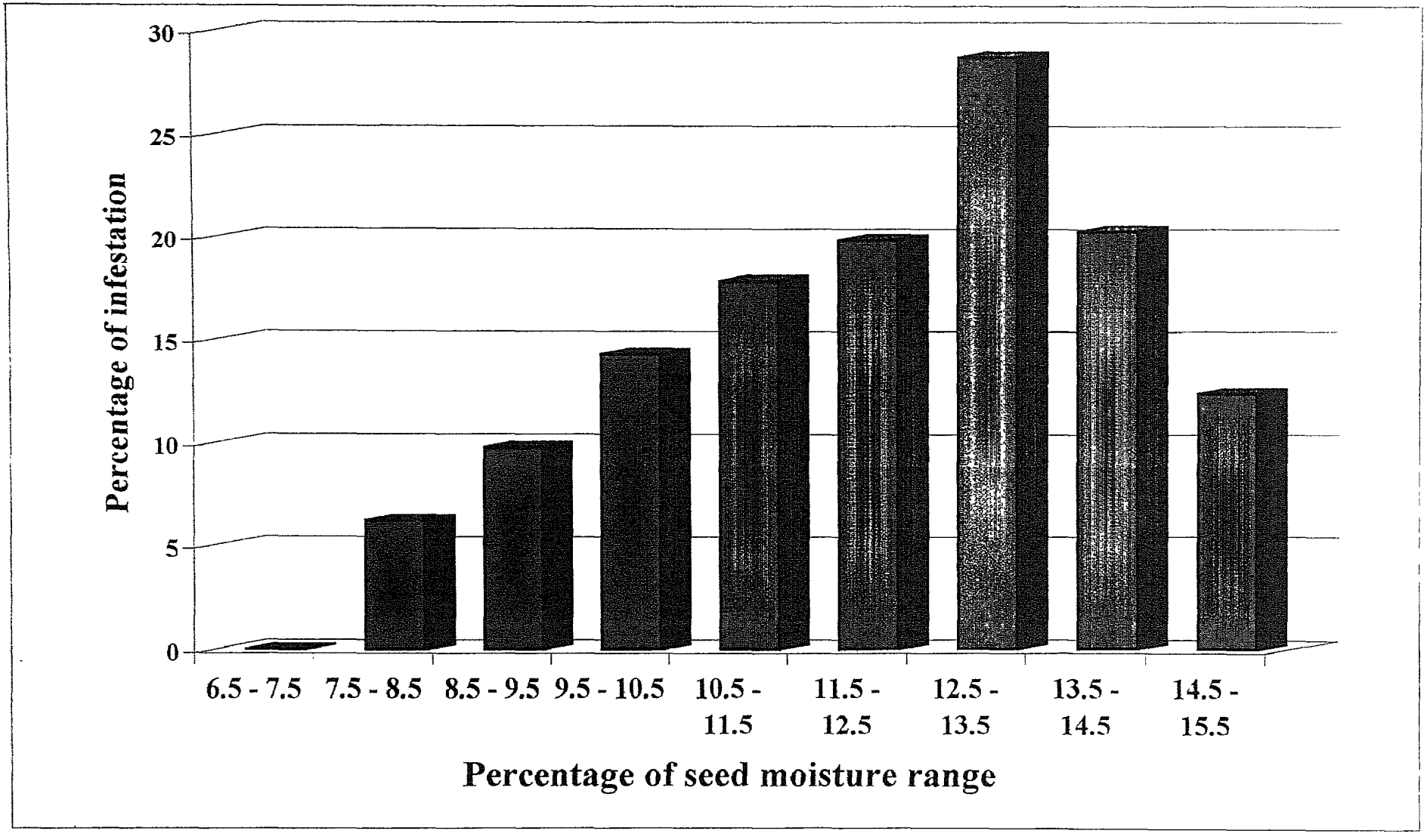
Table - 15 Showing effect of threshing and storage condition on seed infestation of Linseed (*Linum usitatissimum* L.)

THRESHING METHODS:	**Percentage of seeds infestation**
1. Machine	23.00
2. Bullock	14.00
3. Hand threshed	10.00
COLLECTION SOURCES:	
1. Tin container	11.00
2. Kathila	13.00
3. Underground pits	16.00
4. In bags covered with wheat straw	11.00
5. On floor	16.00
6. Earthen pots	16.00

Table - 16 Showing percentage infestation of seeds of linseed (*Linum usitatissimum* L.) at different range of moisture

Percentage of seed moisture range	Percentage of infestation
6.5 - 7.5	0.00
7.5 - 8.5	6.25
8.5 - 9.5	9.82
9.5 - 10.5	14.34
10.5 - 11.5	17.89
11.5 - 12.5	19.92
12.5 - 13.5	28.78
13.5 - 14.5	20.35
14.5 - 15.5	12.45

Percentqge of infestation of seeds of linseed (Linum usitatissimum L.) at different range of moisture



CHAPTER – 7

CONTROL AND INTER-RELATIONSHIP STUDIES

CONTROL AND INTER-RELATIONSHIP STUDIES

To prevent linseed crop diseases effectively, a knowledge of their nature and cause is fundamental to successful control. Once the causal agent has been correctly diagnosed then it is possible to prescribe effective methods for its control. However, they may be prevented by a combination of plant protection principles that are necessary for effective protection against the several diseases causing agencies. The following methods are effective to control the crop diseases caused by fungi, bacteria, nematodes, viruses and nutritional deficiencies:

(i) Adopting appropriate cultural practices, which would either enable a plant to escape from disease or reduce the inoculum potential of pathogen.

(ii) Restricting the movement of diseased crop material from one region to another through legislation.

(iii) Growing disease resistant varieties.

(iv) Using chemicals to save the plant from disease.

The word fungicide has originated from two Latin words, viz. fungus and caedo. The word caedo means 'to kill'. Thus a fungicide

would be any agency which has the ability to kill the fungus. The fungicides are just one of the weapons in our arsenal to Defence against unwanted diseases of fruit plant. Fungicides may work alone or may be used in combination to prevent plant diseases. Once a disease becomes established in orchard, it is generally difficult to eliminate. For this reason, fungicides have received particular attention from research workers and the agro-chemical industries. A number of different fungicides have been developed for plant disease control during last few years.

A seed borne pathogen has greater capacity for spreading into growing crops than pathogens other than seed borne. As the primary infection comes from the infected seeds, experiments were carried out to control seed borne fungi by various fungicides.

The fungicides were evaluated on the basis of their inhibitory effect on the vegetative hyphae, by the method suggested by Foreberg (1949). Small pieces of sterilized cotton threads (about 1.5 cm in length) were placed in Petridishes containing Asthana and Hawker's medium 'A' and were separately inoculated with different Fusaria. The threads, which got covered with vegetative hyphae of the various fungi within a week, were subsequently rolled or dipped in the fungicides. The treated threads were transferred to another set of Petridishes containing above medium and were incubated at 25 ± 1°C for 7 - 10 days. Those fungicides, which did not allow pathogens to grow, were considered effective against a particular fungus. Results of the preliminary trials were recorded in Table - 17.

On the basis of preliminary study, ten fungicides viz., *Agallol, Agrosan,* Benlate, Brassicol, *Captan,* Cercobin, Farbam, Hinosan, TBZ, and *Vitavax* were found effective. They were further tried on seeds of *Linum usitatissimum.* Three concentrations viz. 500, 1000 and 1500 ppm solution was prepared in sterilized distilled water in sterilised flasks and seeds were treated with each concentration.

Flasks containing seeds were shaken well and kept for a few hours. Treated seeds were placed in Petridishes containing PDA and incubated at 25 ± 1°C for a week to allow the growth of fungi. Treating the seeds with sterilized distilled water made suitable controls. In another set of experiment, treated seeds were placed on the sterilized moistened blotting papers. Results are recorded in Table - 18.

Several workers worked on the control of oilseed diseases. To be named, Kumar and Khare, 1970; Anahosur *et al.,* 11973; Chohan and Singh, 1973; Daftari and Verma, 1973; Havare *et al.,* 1973; Shukla and Singh, 1973; Agarwaal *et al.,* 1974; Sharma and Kaistha, 1974; Singh *et al.,* 1974; Ellis *et al.,* 1975; Jhamaria *et al.,* 1975; Vyas and Nene, 1975; Bol Kan *et al.,* 1976; Maggione and Lam-Sanchez, 1976; Tu and Chang, 1976; Oshii, 1977; Sharma, 1981; Prasad and Singh, 1983; Kumar and Singh, 1984; Rai and Singh, 1986.

It is evident from Table - 17, that Agallol, Agrosan-GN, Benlate, Ceresan, Cercubin, Farbam. Hinosan, TBZ and Vitavax completely inhibited the growth of all the pathogenic fusaria in the laboratory while Dithane Z-78, Difolatan, Thiram and Kirticopper, were unable to

check their growth. Brissicol, Blitox-50 and Sulphur dust were also found effective against all the pathogens except *Fusarium oxysporum.* Captan was proved to be ineffective against *Fusarium solani* while Plantvax was unable to check the growth of *Fusarium equiseti.*

Results from Table-18 clearly show that all the effective fungicides used for seed treatment reduced the fungal population as compared to untreated seeds (control). It was further noticed that use of these fungicides on seeds did not cause any harmful effect on their germinability.

EFFECT OF LEAF EXTRACTS OF VARIOUS MEDICINAL PLANTS

Effects of some medicinal plants like Neem, Tulsi, Garlic, Onion, Madar, etc. have been reported to possess chemicals, toxic to various micro-organisms and serve as chemical protective barrier to the infection, anti-fungal property of several plant extracts has been reported by various workers.

Fungi reproduce and spread mainly by spores. Spores are minute separable bodies with a special form characteristic of the particular species. The commonest type of spore produced by the fungi is asexual.

Germination of fungal spores is essentially a process during which the normal metabolic and physiological activity is restored after dormancy. According to Gottlieb (1964) germination is the process by

which a spore is transformed from a dormant state of low metabolic activity to one of high metabolic activity. Formation of the germ tube is the outward and visible sign that the metabolic change is complete.

He further stated that an alteration should be brought in the conventional concept that spore germination is the process involving the absorption of water with consequent swelling of the spores, causing the cell wall to rupture with the formation of germ tube. These processes are the visible manifestation of a series of complex metabolic changes, which take place decrying the germination of spores. They are dormancy, maturity, longevity, temperature, hydrogen-ion concentration, water and nutrients etc., which inhibit or accelerate the germination of spores. Water is essential to activate the metabolic activities. The enzymatic activity and the spore germination increases when temperature is near to optimum. Thus the germination of the spores is the result of the action of all the influencing factors operating at the same time.

Spores are known to be more sensitive to environment than mycelium, hence it was considered necessary to investigate the effect of leaf extracts of some medicinal plants on the germination to spores of present fungi at room temperature (25 ±1°C). Ten days old cultures of the three fusarial pathogens, *viz. F. solani, F. oxysporum, F. equiseti* have been used.

Leaves of several plants have been reported to possess chemicals, toxic to various micro-organisms and serve as chemical protective barrier to the infection.

The present work deals with the effect of leaf extracts of a number of medicinal plants on the spore germination of the fungi under study. Spore suspensions were made in the supernatant extract at two concentrations viz. 100 percent and 50 percent. The percentage germination of the spores was recorded after their initial time of germination in Table - 19.

It is evident from the table that out of five medicinal plant tried, leaf extract of Neem (*Azadirachta indica*) at 100 percent concentration completely checked the spore germination of all the three fungi, while 100 percent garlic bulb extract (*Allium sativum*) spores of *F. equiseti* could only germinate upto 20 percent. Next of Neem and Garlic extracts, Ocimum leaf extract (100 percent) exhibited anti-fungal property where spores of *F. solani, F. oxysporum* and *F. equiseti* could germinate upto 32, 18 and 42 percent respectively. In comparison to the above leaf extracts, spore germination of *F. solani, F. oxysporum* and *F. equiscti* was 92, 96 and 92 percent respectively in control sets (distilled water).

Different workers investigated the effect of leaf extracts of various medicinal plants on spore germination of pathogenic fungi. Shekhawat and Prasad (1971) tried leaf extract of *Melia azadirachta, Ocimum sanctum* and *Allium sativum* against 41 species of pathogenic fungi, out

of which *Curvularia Penicillium* and *Helminthosporium spp.* were found unable to germinate on *Melia* and *Ocimum* leaf extracts. Mishra *et al.* (1974) reported complete inhibition of *Curvularia lunata* and *Helminthosporium graminicola* in leaf extracts of *Melia and Ocimum* respectively. Khanna and Chandra (1972), Shukla (2000), Dwivedi and Shukla (2001) have also reported similar observations for the fungi studied by them.

RESISTANCE VARIETIES

The promise of resistance to pests and diseases has attracted the attention of plant breeders ever since the demonstration near turn of the century that resistance could be selected and that it is often simply inherited. The built in protective resistance effective throughout a crop plant life offers a compelling alternative to fungicides and insecticides. In the early years, minimising the use of protective chemicals was desirable because it saved money but today there is an added advantage of reducing their hazard against those pests and diseases such as cereal rusts, soil borne smuts, wilt seed borne and certain nematode disease. There is considerable literature available on the breeding and inheritance of disease resistance in plants. A list of papers published on the inheritance of disease resistance in plants upto 1934 has been given by Hanseen (1934).

The literature, on the genetics of disease resistance in vegetables has been reviewed by Walker (1965) and on field crops by Ausemus

(1943) and Dickson (1956). Inheritance of resistance to viral disease has been reviewed by Halmes (1954), resistance to rust by Hooker (1967) and to nematodes by Hare (1965). Some more recent review articles are those of Hooker and Saxena (1971), Roane (1972) on trends in breeding for disease resistance in crops. Hooker (1974) on cytoplasmic susceptibility in plant disease and Sadasivan (1975). Knott and Dvorak (1976) have discussed alien germplasm as a source of resistance to disease. A recent article is by Browning *et al.* (1977) on managing genes epidemiological and genetic concepts.

The use of disease resistance varieties for controlling plant diseases has been termed as the painless method because it does not cost the farmers anything. The resistant plant defends itself against a potential pathogen by means of a number of physical and chemical characteristics of the plant or which are formed in the plant in response to infection. The physical characteristics act as mechanical barriers, which prevent the entrance and spread of pathogen in plant. The chemical factors, which are toxic to the pathogen, inhibit its growth and activity in the plant.

Since Biffen's (1905) elucidation of the inheritance of the resistance in single Mendelian fashion, spectacular progress has been made in our understanding of the genetic aspects of parasitism and disease resistance. The mechanisms of variability that make the pathogens versatile in their behaviour and host range are now well known.

Flow (1955) explained host parasite interaction in linseed rust by assuming gene for gene-relationship between rust reaction in the host and pathogenicity in the parasites.

Hart (1926) observed that the stomata of rust resistant wheat remain closed till late in the morning. By the time they open, the germ tubes of uredo-spores, formed earlier in the dew, get killed due to evaporation of the water. Link and Walker (1933) reported presence of protocatecuic acid and catechol in the dry Pigmented scales of onion bulbs resistant to *Colletotrichum circinans*. Timonin (1940) reported that varieties of flax excrete hydrocyanic acid (HCN) in the rhizosphere.

Orton (1900) obtained resistant cotton variety from selection and multiplication of resistant individually. He observed that some cotton plants did not show wilting in the heavily infected crop. He collected seeds from these plants and planted them in wilt infested soil. By several such plantings he ultimately got most resistant plants which grew well on heavily infected soils. A vast majority of crop varieties can be attacked by a single pathogen or many different kinds of pathogens can attack a single variety. Most plants are naturally resistant to many pathogens. During evolution of plant life weak and disease susceptible individuals have been progressively eliminated by nature and the plants which exist today are those having developed resistance to most pathogens in a particular geographic area.

Selection for horizontal resistance has been utilised consciously or unconsciously as long as agriculture has existed, but in recent years

horizontal resistance has become an object in itself for systematic breeding.

In the present study high yielding varieties of *Linum usitatissimum* L. (Pantnagar, Mukta, R - 17, N - 55 and Neelam) have been tested against seed borne pathogens.

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the test isolates and planting them in pots filled with sterilized field soil. In another set of experiment, healthy seedlings were planted in pots containing infested soil with 3 percent maize meal inoculation. The experiments were continued for 2 years (1999 - 2000). Healthy seeds of the above varieties harvested in first year of production were sown the next year. The results are summarised in Table -20.

Results from the experiments clearly show that out of five high yielding varieties of linseed (*Linum usitatissimum* L.) crop tested against *Fusarium oxysporum, Fusarium solani* and *Fusarium equiseti,* N - 55 and Neelam were found resistant to the pathogens under investigation.

INTER-RELATIONSHIP STUDIES

The toxins produced by the seed-borne fungi are known to suppress germination of seeds (Christensen and Drescher, 1954 and Mishra and Singh, 1969). Sullia (1966) observed suppression of

germination in leguminous seeds soaked in the filtrate of *Aspergillus niger*. Armolic *et al.* (1956) reported that the culture filtrate of *Fusarium moniliforme* was highly toxic to barley grains even after 10 days' growth.

The seeds in turn also play some part in regulating the physiological set up of the fungi, associated with them. Seed-coat leachates have been reported to cause inhibitory effect on certain seed fungi (Ark and Thomson, 1958; Srivastava and Mishra, 1971 and Mishra and Kanaujia, 1973).

The inter-relationship between seed and seed fungi is very complex. A thorough study is needed to understand the phenomenon. In this chapter an attempt has been made to investigate the inter-relationship between linseed and their seed borne fungi.

Seed samples of *Linum usitatissimum* L. were collected from different places. They were brought to laboratory in sterilized containers and similar types were mixed together to get a composite sample.

For the study of the effect of seed coat leachates on the spore germination of the *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Alternaria, Cladosporium, Curvularia, Fusarium, Penicillium* and *Rhizopus*. 10 gm surface sterilized seeds from each composite sample were taken in 250 ml conical flasks. They were soaked in 100 ml of sterilized double distilled water for 24 hours. The flasks were shaken gently for 30 minutes. The water from the flasks

were separately filtered and concentrated to a volume of 10 ml on water bath (Mishra and Kanaujia, 1973).

The seed extract was prepared by crushing 10 gm of surface sterilized seeds in 100 ml of sterilized double distilled water. The filtrate obtained was known as seed extract. The germination of fungal spores was studied by hanging drop technique described by Hoffman (1860). The effect of seed coat leachates and seed extracts on spore germination of above fungi are presented in Table - 21.

The effect of 15 days old culture filtrate of *Fusarium oxysporum, Fusarium solani* and *Curvularia lunata* on seed germination of Linseed was studied by soaking the surface sterilized seeds in culture filtrates of different concentrations (20, 40, 60, 80 and 100 percent) for 24 hours. The treated seeds were then placed on petridishes containing sterilized moist blotting papers. Control sets were also maintained by soaking seeds in sterilized distilled water and placing them on sterilized moist blotting papers in petridishes. In each case, 100 seeds per treatment were taken. The number of seeds germinated was counted after 60 hours.

The effects of 15 days old culture filtrate of *Fusarium oxysporum, Fusarium solani* and *Curvularia lunata* on seedling growth of linseed were also studied. Roots of two seedlings (15 days old) were dipped for 24 hours in test tubes containing culture filtrates. Triplicate sets were used for each treatment. The seedlings were then removed and were

planted in pots containing sterilized field soil. Results of the above experiment are presented in Table - 22.

Results from Table - 21 show that percentage germination of present fungi was notably effected when placed in seed coat leachates and seed extracts of linseed. On the other hand in general a high percentage of spore germination of above fungi was recorded in sterilized double distilled water.

It is evident from the Table - 22 that the germination of Linseed seeds was affected considerably, when treated with different concentrations of culture filtrates of present organisms. The concentrations above 20 percent caused a considerable decrease in the seed germination of linseed. The reduction in seed germination was more pronounced in culture filtrates of *Fusarium oxysporum.* In all the cases increases in the concentration of filtrate lowered the germination percentage of seeds. In control sets, however, the percentage seed germination was very high and it varied from 94 to 98 percent.

It was observed that 15 days old culture filtrates of *Fusarium oxysporum, Fusarium solani* and *Curvularia lunata* affected the seed germination of linseed. Concentrations of culture filtrates above 20 percent inhibited the germination of seeds considerably.

It is well known that the fungi affect the seed germination directly either by lowering the viability of the seed, by making it nutritionally poor or by secreting certain mycotoxic substances unfavourable to the seeds.

There was a marked effect of culture filtrates on the growth of seedlings. The culture filtrates almost checked the growth of seedlings, when they were planted in pests. Their growth was not vigorous as compared to control plants. In another set of experiment in which plants (15 days old) were kept in culture filtrates (in 150 ml conical flask) under laboratory condition for 7 days. It was observed that roots became pale and in a few cases localised dark brown spots developed. While plants kept in sterilized distilled water, no change was observed and they remained healthy.

Seed extracts and seed-coat leachates of linseed exerted influence on the spore germination of all the dominant members of the seed mycoflora, but the magnitude of influence varied with the forms. Seed extract had the maximum effect (74.73 percent) reduction in spore germination. On the spore germination of *Cladosporium* followed by *Alternaria* spp. (53.30 percent), *Alternaria flavus* (49.70 percent) and *Rhizopus* spp. (49.20 percent). Seed coat leachates were comparatively less effective as it showed pronounced effect on the spore germination of only *Cladosporium spp.* and *Penicillium spp.* Other fungal forms were affected to a lesser degree by both extract and leachates and showed more than 50 percent spore germination in their presence.

This may probably due to presence of some anti fungal substances in the seed coat leachate. These substances may play an important defensive role against seed infection (Ark and Thompson, 1958 and Srivastava and Mishra, 1971).

Table - 17 Evaluation of fungicides

Fungicides	Chemical nature	*F.equiseti*	*F. oxysporum*	*F. solani*
Agallol	Methoxyothyl mercury chloride	-	-	-
'Agrasan' GN	Phenyl mercury acetate and ethyl mercury chloride	-	-	-
Benlate	Methyl 1 - (butylcar-bamyol) 2 - benzimi-dazolecarbomate	-	+	-
Brassicol	Pentachloronitro benzena ((PCNB)	-	-	-
Blitox-50	Copper oxychloride	-	-	-
Captan	N-(Trichloromethuyl) thio-4-cyclohexene -1, 2 - dicarboximide	+	+	-
Cersan	Phenyl mercury acetate	-	-	-
Difolatan	N (1,1,2,2, tetrachloro-ethyl) Sulfenyl-cis-4-cyclohexene - 1, 2 - dicarboximide	+	+	+

Fungicides	Chemical nature	*F.equiseti*	*F. oxysporum*	*F. solani*
Dithane Z-78	Zinc ethylene bisdithiocarbamate	+	+	+
Farbam	Ferric dimethyl dithio carbamate	-	-	-
Hinosan '50'	O-ethyl-S, S-diphenyl Phosphodithioate	-	-	-
Plantavax	2, 3-Dihydro-5-carboxanilido-4-methyl 1, 4Oxathiin-4, 4-dioxide	-	-	+
Sulphur	Sulphur	-	-	+
TBZ	Thiabendazole (2-4-thiazolyl) benzimidazole	-	-	-
Thiram	Bis (Dimethyl thiocar-bomyl) disulphate of tetra methyl-thiuram disulphide	+	+	+
Kirti copper WXP 50	Copper oxychloride	+	+	+

Fungicides	Chemical nature	*F.equiseti*	*F. oxysporum*	*F. solani*
Vitavax 75	2, 3-Dihydro-5-Carboxanilido-6-methyl -1.4-oxathiin	-	-	-
Cercobin	Benzene thiophanate	-	-	-
Control	-	+	+	+

+ shows presence of the fungal growth

Table - 18 Effect of different fungicides on fungal flora associated with *Linum usitatissimum* L. seeds

Fungicides	Conc. in ppm	% seeds showing infestation after treatment with fungicides					
		A. flavus	*A. niger*	*F. solani*	*F. oxysporum*	*F. equiseti*	*Macro-phomina phaseolina*
Agallol	500	-	-	4	1	4	-
	1000	-	-	3	2	3	-
	1500	-	-	3	3	2	-
Agrosan	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	1500	-	-	-	-	-	-
Benlate	500	-	-	2	2	2	-
	1000	-	-	1	-	1	-
	1500	-	-	1	-	1	-
Brassicol	500	-	-	4	-	3	-
	1000	-	-	5	-	1	-
	1500	-	-	3	-	-	-
Captan	500	-	1	-	-	-	1
	1000	-	-	-	-	-	-
	1500	-	-	-	-	-	-

Fungicides	Conc. in ppm	% seeds showing infestation after treatment with fungicides					
		A. flavus	*A. niger*	*F. solani*	*F. oxysporum*	*F. equiseti*	*Macro-phomina phaseolina*
Cercobin	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	1500	-	-	-	-	-	-
Farbam	500	-	2	1	-	-	-
	1000	-	2	-	-	-	-
	1500	-	-	-	-	-	-
Hinosan	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	1500	-	-	-	-	-	-
TBZ	500	1	-	1	5	3	-
	1000	-	-	1	2	1	-
	1500	-	-	-	3	1	-
Vitavax	500	1	-	2	1	-	-
	1000	1	-	-	1	-	-
	1500	-	-	-	2	-	-
Control	-	3	2	5	4	4	2

Table - 19 Showing effect of leaf extracts of various medicinal plants on spore germination of *Fusarium* species population

Sl. No.	Leaf extracts	Concentration (%)	Percentage of germination		
			F. solani	*F. oxysporum*	*F. equiseti*
1.	*Strychnos nuxvomica* L.	100%	33	24	43
		50%	64	42	81
2.	*Calatropis procera* (Air) R. Br.	100%	35	24	47
		50%	57	48	72
3.	*Azadirachta indica A. Juss*	100%	-	-	-
		50%	22	13	42
4.	*Ocimum sanctum* L.	100%	32	18	42
		50%	60	40	62
5.	*Allium sativum* L.	100%	-	-	-
		50%	31	27	63
6.	Control	-	92	96	92

Effect of leaf-extract of various medicinal plants on spore germination of *Fusarium* species population

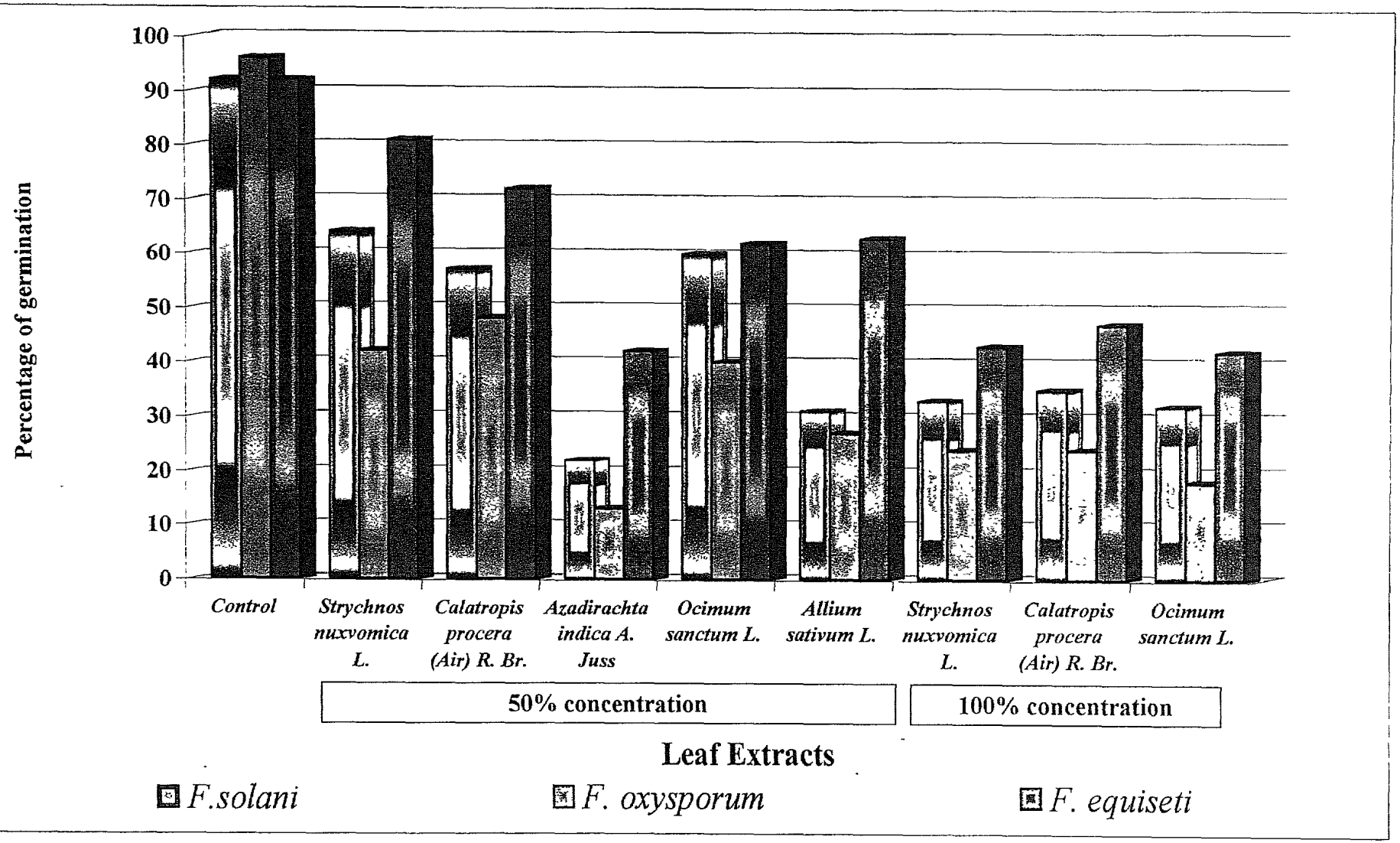


Table - 20 High yielding varieties of linseed (*Linum usitatissimum* L.) crops showing percentage infection of three *Fusarium spp.* in two successive years (1999 - 2000)

Year 1999

Varieties	% infected plants		
	F. oxysporum	*F. solani*	*F. equiseti*
Pantnagar	16	13	16
Mukta	03	04	03
R - 17	06	03	04
N - 55	05	15	00
Neelam	00	00	00

Year 2000

Varieties	% infected plants		
	F. oxysporum	*F. solani*	*F. equiseti*
Pantnagar	*13*	50	14
Mukta	04	08	02
R - 17	06	03	04
N - 55	00	00	00
Neelam	00	00	00

Table - 21 Effect of seed extract and seed coat leachates of linseed (*Linum usitatissimum* L.) on spore germination of seed borne fungi

Fungal isolates	Percentage spore germination				
	Seed extract		Seed coat leachates		Control (in water)
Aspergillus fumigatus	64.10	(32.03)	58.55	(37.91)	94.30
Aspergillus flavus	50.30	(49.70)	65.55	(34.45)	100.00
Aspergillus niger	61.80	(38.20)	67.80	(32.20)	100.00
Alternaria spp.	40.30	(53.30)	66.10	(23.41)	86.30
Cladosporium spp.	20.80	(74.73)	37.10	(54.92)	82.30
Curvularia spp.	61.80	(35.62)	73.15	(23.80)	96.00
Fusarium spp.	57.55	(42.45)	59.20	(40.80)	100.00
Penicillium spp.	71.30	(22.50)	37.80	(58.91)	92.00
Rhizopus spp.	50.80	(47.20)	54.80	(43.20)	100.00

Effect of seed extract and seed coat leachates of Linseed (*Linum usitatissimum* L.) on spore germination of seed-borne fungi

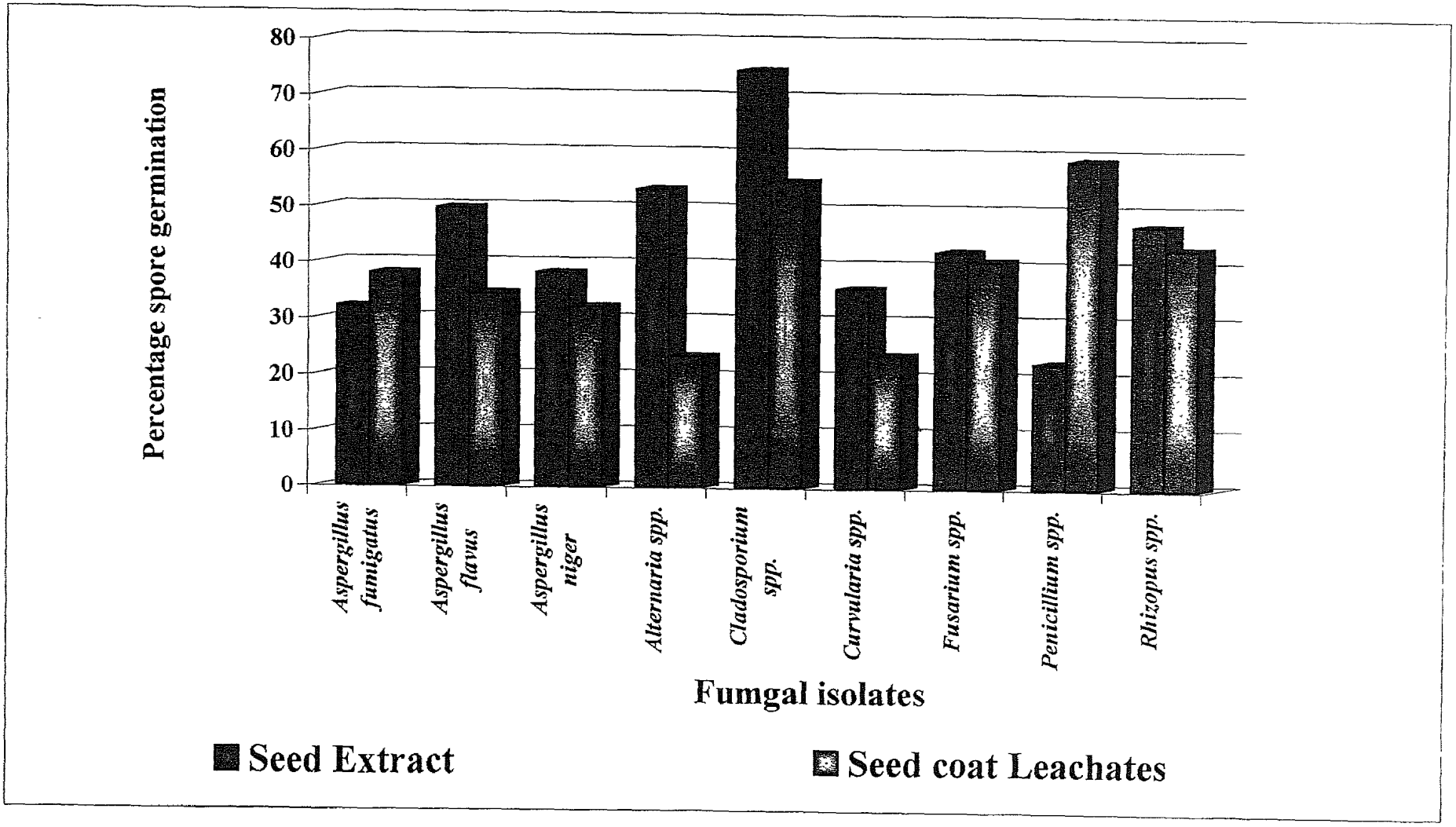
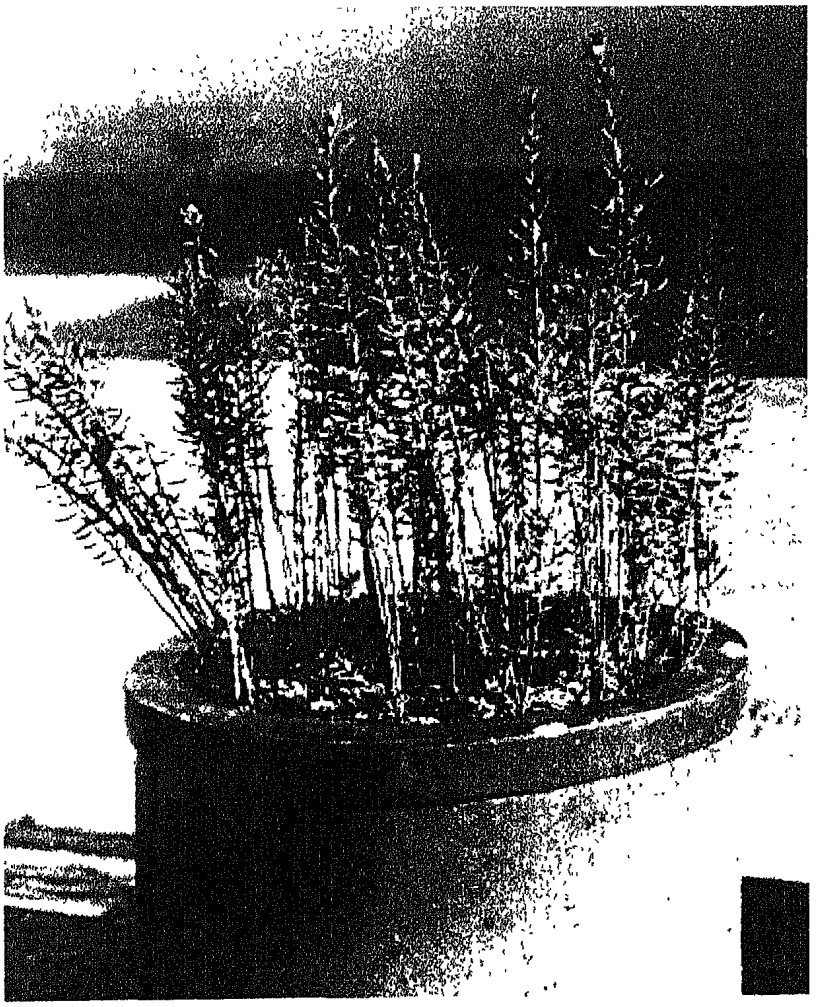


Table - 22 Effect of culture filtrates of *Fusarium oxysporum, Fusarium solani* and *Curvularia lunata* on the seed germination of linseed (*Linum usitatissimum* L.)

Pathogen	Concentration of culture filtrate	Percentage seed germination	Control (distilled water)
Fusarium oxysporum	20	85	94
	40	70	95
	60	50	96
	80	40	97
	100	40	98
Fusarium solani	20	90	94
	40	80	95
	60	69	96
	80	60	97
	100	55	98
Curvularia lunata	20	88	94
	40	80	95
	60	65	96
	80	53	97
	100	45	98

CHAPTER – 8

DISCUSSION AND CONCLUSION



DISCUSSION AND CONCLUSION

"People need food, not production statistics, and a crop is not food until it is eaten. A programme to reduce storage losses, probably could result in a 10-20 percent increase in available food now in some of the developing countries, and might also assure that whatever increase occur in production in the future would be used for the nourishment of people, not for feeding pests"

- Christensen and Kaufman, 1869.

At present India is facing acute shortage of oil crops inspite of large areas of their cultivation. Seed is the basic unit for crop production and farmers clamour for a few seeds of a new of hybrid variety. However, while introducing seeds and other plant materials from various countries, we have to be very careful, so that no serious pathogens are introduced. Due to increase in demand of healthy seeds in developing disease free crop it has become obligatory on the part of Phytopathologists, to know "Seed Pathology". Neergaard (1977) has

very critically and vividly written a textbook on this subject in two volumes. In addition to actual losses due to other hazards, high percentage of losses are recorded as a result from seed-borne diseases. These include reduction

(I) in actual value of the produce for human use

(II) in the germination percentage of seeds, which may render seeds unfit for seedling purpose

(III) deterioration of produce in storage and

(IV) harmful effects on human or livestock.

Realising the possible threat from seed-borne diseases, we also need to approach the problem on scientific lines and precise study dealing with important aspects of seed health and seed testing.

Various aspects of seed-borne pathogens have been discussed by Nobel (1951, 1957); Nobel *et al.* (1958); and Dykestra (1961). Host parasite relation and environmental influences in seed borne diseases have been discussed by Wallen (1964). Nene and Agarwal (1978) have given information on some seed-borne diseases and their control. Suryanarayan (1978) gives a comprehensive account of the currently important seed-borne pathogens are of two types:

(I) Adherent to the outer covering of the seed; and

(II) Borne inside the seed.

Seeds of linseed crops may often be expected to carry the infection of fungi in the embryo. Seed coat is the common site of infection of most seed transmitted fungi imperfecti.

The studies on isolation of mycoflora from linseed seeds yielded some interesting results. In all, twenty-two fungal species were recorded from linseed crop seeds. Seeds collected from various places (Allahabad, Banda, Hamirpur, Mahoba, I. A. R. I. New Delhi, Allahabad Agricultural Institute - Allahabad, G. B. Pant University of Agriculture and Technology - Pantnagar and from local markets) did not show much variation in their fungal flora, although their frequencies were different in each sample. It was also observed that seeds collected in humid weather conditions (July to October) were heavily associated with the species of *Aspergillus, Penicillium* and *Rhizopus.*

The rate at which seed deteriorates in storage is influenced greatly by a number of factors, of which the most important appear to be moisture content, temperature, the kinds of fungal flora involved, the length of storage period, storage containers and the rate at which the fungal grow, the condition of seed (Physical and biochemical) and the number and perhaps the severity of injuries on the seed coat of the seed.

The longevity of seed kept in storage is predominantly dependent on the moisture content of the seed and on the temperature and relative humidity in the storeroom. Toole (1942) and Harington (1972) indicated approximate percentage of moisture content in various kinds of seeds as related to different degrees of relative humidity of the air. Seeds high

in oil content will have much lower moisture content than seeds high in protein in starch content. Moisture level, obviously favours rapid growth and multiplication of fungi. For each of the common species of storage fungi, there is minimum moisture content in seeds below that it can not grow.

In the present study, moisture content below 8.5% of all the seeds, showed absence of any fungal growth. It was interesting to note that as the moisture level was increased above 8.5%, fungal invasion was also increased. At higher moisture percentage (12.5 - 13.5 percent) heavy invasion by species of *Aspergilli* was observed. In a few cases species of *Rhizopus* were also observed.

Storage studies revealed that seed samples stored upto 12 months were heavily associated with both storage and field fungi and further upto 2 ½ years of storage it decreased. It was further noticed that machine threshed seeds were more amenable to fungal flora than bullock threshed seeds, possibly due to more injury in the former case (Kaulik, 1973; Justice, O. L. *et al.*, 1978).

Dicotyledonous seeds are reported by various workers to be particularly sensitive to thresher injury which in beans may produce upto 30 percent abnormal seedlings (Harter, 1930).

Troelson (1947) reported that in humid conditions seeds are easily damaged by hard threshing, when the moisture content of the seed was high. Flaxseed was also sensitive to thresher injury. The seed

coats are brittle and may easily rupture when the seeds are knocked against a hard surface (Kommedahl *et al.*, 1955).

Isolation from seeds collected from various containers showed that tin containers and sack kept in wheat straw were associated by a fewer fungi than seeds stored in earthen pots and on floor.

Shands (1937) found that *Fusarium graminearum* could live in barley upto 27 months. Christensen (1963) recorded 20 months, *F. culmorum* and *F. avenaceum* were viable for even a shorter period of time (Shands, 1937) whereas Panchet (1966) recorded survival of *F. nivale* although in low percentage, in wheat after 42 months. Dungan and Moehler (1944) recorded that in maize, *F. graminearum* died out completely in 2 years, most of the seed being free of the pathogen already after 15 months. On the other hand, some of the seeds still harboured viable *F. moniliforme* after 8 years. *F. oxysporum* in seeds of red clover could still be isolated after 6 years of storage in air-tight containers at 5 percent relative humidity of the air (Narkiewicz-Jodko, 1974). Many species of *Alternaria* commonly encountered in seed are long lived under storage of the seed usually at least 5 - 6 years (Neergaard, 1945). Christensen (1963) found in barley, *Alternaria tenuis* viable after storage for 6 years.

Lutey and Christensen (1963) found substantial reduction in percentage of field fungi such as *Alternaria, Drechelora* and particularly *Fusarium* in barley kernels kept for a few months at 14 percent moisture content at 20°C.

The fungal isolates obtained from the above studies were screened for pathogenicity and among the fungal flora isolated from linseed crop. Six species of *Fusarium* and *Rhizopus,* two species of *Allternaria* and one species of *Alternaria* and *Macrophomina* were found to be pathogenic on their respective hosts. The nature of diseases observed were, foot rot, flower-rot, head-rot, leaf spot, root-rot, seed-rot, seedling blight, stem-rot and wilt.

There are reports by Chattopadhaya and Sengupta (1955), Chattopadhaya and Basu (1957) and Grewal *et al.* (1974) of *Fusarium solani* causing wilt in *Psidium guajava, Abelmoschus esculentus* and *Cicer arietinum* respectively. Kerr (1963) also reported root-rot and *Fusarium* wilt complex of pea to be caused by *F. solani f. spp pisi. Fusarium oxysporum* is reported by various workers as wilt causing pathogen of many hosts and most of these are seed-borne (Noble and Richardson ,1968 and Anderson, 1974).

F. equiseti has been found quite frequently in seeds of many hosts (Ram Nath *et al.,* 1970 and Anderson, 1974). It may cause stem -rot and foot-rot of the plants. *F. moniliforme* is known as causal agent of seedling blight, foot-rot, stunting and hypertruphy (Booth, 1971) and as seed-borne (Noble and Richardson, 1963; Ram Nath *et al.,* 1970 and Anderson, 1974).

Alternaria alternata was responsible for causing the leaf-spot diseases of their respective hosts at different stages of plant growth. *A.*

alternata is reported by Groves and Skilke (1944) and Neergaard (1945) on different seeds as saprophyte.

Macrophomina phaseolina is reported as causal agent of charcol-rot, stem-rot, ashy stem-blight (Noble and Richardson, 1968; Watanabe, 1972 and Chidambaram and Mathur, 1975).

Rhizopus arrhieus and *R. stolonifer* have been reported by Venkatram (1950) and Halisky and Satour (1964), as part of boll-rot organism of cotton, leading to internal infection of seed. *Trichothecium roseum* is a common saprophyte on seeds (Doyer, 1938 and Malone and Maskett, 1964).

Benoit and Mathur (1970) have monographed seed-borne species of *Curvularia* and described their habit on seed and reviewed their occurrence as pathogens mainly on cereals. Mathur *et al.* (1973) reported *Chaetomium globosum* on seeds of *Pennisetum typhoides.*

The microbial infestation of seeds after harvest cause development of many physical, biochemical and physiological deterioration in seed. The rate of deterioration increased with the poor and improper storage conditions. The micro-organisms growing on seeds secrete metabolites which cause biochemical degradation of seed substances. There are many complex seed constituents which are affected due to mouldiness of the seeds. However, in the present studies changes in protein and oil contents of few oil crop seed, have been investigated respectively.

In linseed seeds, slight reduction of oil content was observed. It was noticed that *Fusarium equiseti, F. oxysporum* and *F. solani* were unable to bring about any change in colour and odour of the seeds of *Linum usitatissimum*. This may be due to short incubation period and nature of the above fungi.

Temperature is one of the important environmental factors which play a significant role in governing various metabolic activities. Temperature ranges are which favours mycelial growth, vary considerably depending upon the organism concerned. It was evident from the study that growth, sporulation and chlamydospore formation of the present organisms were prominently influenced by temperature variation. All the three species of *Fusaria* could grow between a range of 10 to 30°C. Their good growth and excellent sporulation were recorded at 25°C. No chlamydospores were found at 25°C except in case of *Fusarium solani*. Chlamydospore formations were better in all the three species of *Fusarium* when the temperature was below and above 25°C.

The hydrogen ion concentration affects permeability of protoplasmic membranes, uptake of minerals, entry of essential vitamins and organic acid into the cell activities of enzyme systems, synthesis and stability of proteins and other life processes. The pH of the medium is changed by the growth of fungus, at the same time pH also change the growth pattern of the organism. A highly acidic or a highly alkaline media inhibits the growth of most of fungi. The growth of three *Fusaria* was maximum at pH 6.0.

C : N ratio may be harmful because it has been observed that in the presence of low carbon supply, a decrease in the population of root pathogens under high C : N ratio in the soil has been demonstrated in several instances. Amendment to provide C : N ratios of 10, 20, 40 and 80 were based on one percent glucose in air-dried soil and sodium nitrate. It was observed that as the C : N ratio was increased, the population of three Fusarium spp. declined in the soil.

Studies on inter-relationship between seeds and seed-borne fungal flora revealed that fungal-spore germination in seed-coat leachates and seed extracts decreased in comparison to control sets. The inhibition was more pronounced in the former. This may be due to presence of some anti-fungal substances in seed coats. It was also observed that culture filtrates of selected organisms checked the seed germination and seedling growth remarkable.

From the above studies, it is clearly evident that the seed mycoflora plays an important role in bringing about the disease development in field. Therefore, it is essential to find out possible control measures for these maladies. The primary screening of 18 fungicides was determined against three *Fusarium* species and among them 10 was found to effective, as they retarded the mycobial growth. The efficiency of affected fungicides was also tested against growth of fungi on seeds, and to control diseases coming on seedlings and plants. It was observed that fungicidal seed treatment reduced the fungal population without any harmful effect on seed germinability. Nene *et al.* (1969); Nene (1971); Nene and Srivastava (1971); Lalithakumari *et al.*

(1972); Chamberlain and Gray (1974); Rati and Ramlingam (1974); Kumar and Singh (1986) have also shown that fungicidal seed treatment have improved seed emergence. Dharam Vir and Grewal (1961) and Dharam Vir *et al.* (1970) have established the superiority of some of these fungicides in control of some seed-borne diseases, where infection is deep seated. Seed-borne infection of *F. semitectum* was controlled by seed treatment (Saharan and Gupta, 1974).

On the basis of data obtained, proper care of seeds of linseed should be taken to prevent fungal deterioration. Necessary precautions should be taken to avoid injury to the seeds. Seeds to be stored should either in tin containers or in gunny bags (sacks) covered with wheat straw.

To save the germs and to check the development of various diseases caused by *Fusarium equiseti, Fusarium oxysporum* and *Fusarium solani,* seeds of *Linum usitatissimum* should be treated with any one of the fungicides, viz. Agrosan, Cercobin, Ferbam and Hinosan at 1500 ppm. Hinosan was found to be more effective.

The host and the period of incubation are very much affected by the amount of moisture available. A novel idea of controlling seed-borne diseases by leaf extracts revealed that leaf extracts of *Azadirachta indica, Ocimum sanctum* and *Strychnos nux vomica* controlled fungal seed contamination. Leaf extracts of the medicinal plants checked the spore germination of the fungi investigated. The possibility of the presence of different chemical compounds, fungicidal or fungistatic in

action in these extracts, which exerted inhibitory influence upon germination of fungal spores, was always there.

While studying effect of leaf extract of medicinal plants on spore germination and disease development, leaf extract of five medicinal plants which were found effective on spore germination of *Fusarium spp.,* Neem (*Azadirachta indica*) at 100 percent concentration, completely checked the spore germination of three *Fusarium spp.* It was further observed that *Allium sativum* leaf extract at 100 percent concentration was also proved effective to check the spore germination of test organisms. Third effective leaf extract was *Ocimum sanctum.*

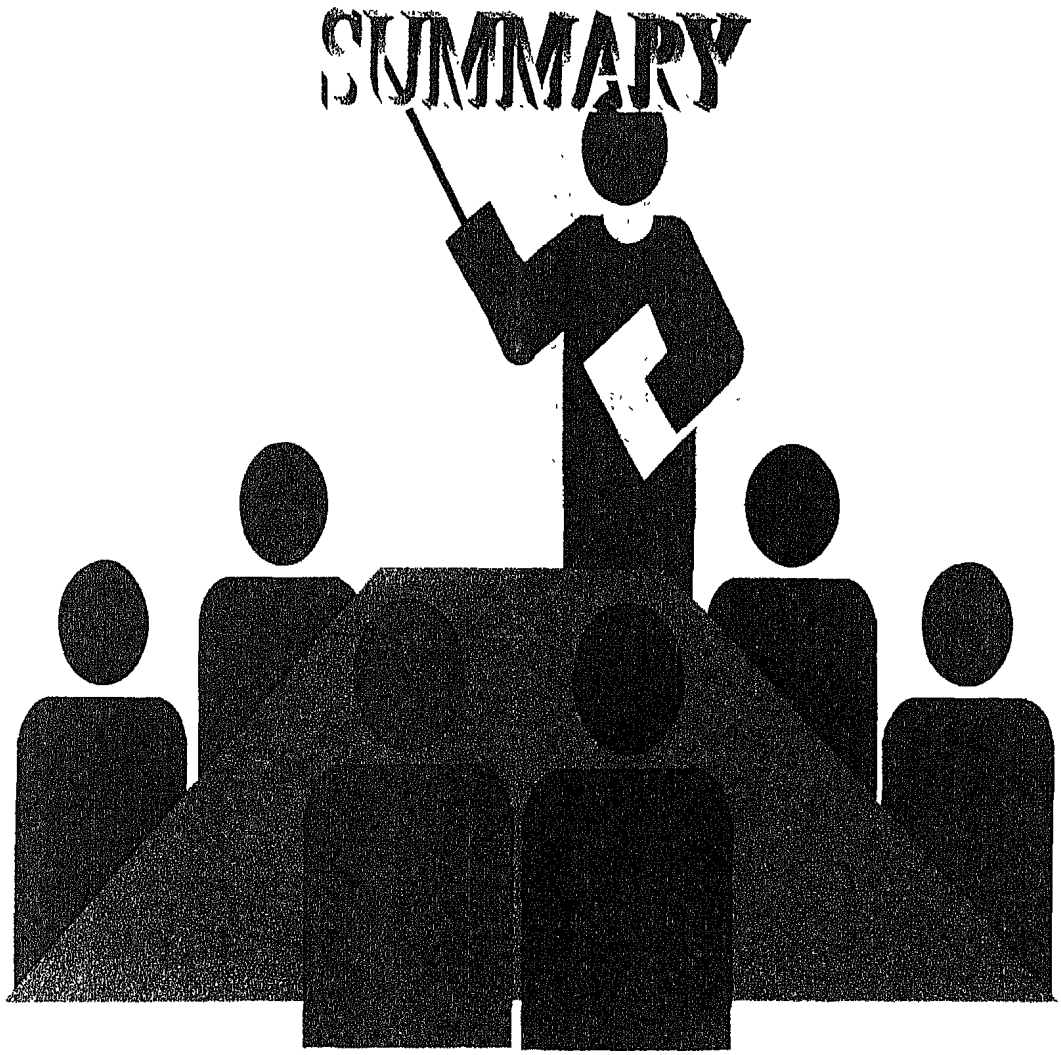
In the present study on high yielding varieties revealed that out of six yielding varieties of linseed tested, two (N-55 and Neelam) were found to be resistant to all the pathogens. Most of the field fungi were sensitive to high temperatures and usually disappeared under such temperature conditions (Lutey and Christensen, 1963). *Alternaria alternata* and *Rhizopus stolinifer,* however developed under temperature above 40°C (Helberg and Kalk, 1972).

Biological control has been broadly defined as the encouragement of beneficial organism already existing in locality or of the introduction of suitable new species of exotic parasitic organisms, which are parasites or harmful pathogens in a locality where the pest in thriving with a view to control the disease. Though the practical application of biological control is of comparatively a recent origin, inter-actions

between different organisms in an eco-system much have been existent for the maintenance of a stable population in an environment.

The effect of 15 days old fungal culture filtrates on seed infestation showed that percentage seed contamination was significantly low as compared to control sets. Seed-coat leachates were also found to inhibit the fungal spore germination and decreased the fungal seed contamination. This was probably due to presence of some anti-fungal substances.

CHAPTER – 9



SUMMARY

Various seed samples of linseed crop were collected from I.A.R.I., New Delhi; Allahabad Agricultural Institute, Allahabad; G. B. Pant University of Agriculture and Technology, Pantnagar and from local markets. Fungi was isolated, purified and maintained on malt extract and Potato Dextrose Agar media, for further examination and pathogenicity tests. Morphological studies were carried out and identifications were made.

In the present investigation an attempt has been made to isolate fungi from different seeds stored for different length of time as well as stored in different containers. An attempt has also been made to isolate fungi from hand, bullock and machine threshed seeds. Effect of seed moisture on seed fungal flora has also been studied.

Seeds of Linseed (*Linum usitatissimum* L.) crop were collected from various places and fungi were isolated, purified and maintained on media. The results recorded as follows:

Fungi isolated from seeds of linseed 'Flax' (*Linum usitatissimum* L.)

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Macrophomina phaseolina (Tassai) Gold	*Aspergillus niger* and *Alternaria alternata*
Alternaria state of *Pleospora infectoria* Fuckel	Fusarium equiseti
Aspergillus flavus Link	*Aspergillus flavus*
Fusarium equiseti (Corda) Sacc.	*Fusarium solani*
Fusarium oxysporum Schlecht.	
Aspergillus terreus Thom.	
Alternaria alternata (Fr.) Keisslerr	
Aspergillus niger Van tieghem.	
Aspergillus fumigatus Fresen.	
Chaetomium arcuatum (Rai and Tiwari)	
Curvularia lunata (Wakker) Beedizn.	

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Drechslera spp.	
Penicillium javanicum Van bemya.	
Rhizopus arrhisus Fischer	
Fusarium solani (Mart.)	
Fusarium semilectum Berk and Curt.	
Fusarium moniloforme Sheld.	
Cladosporium oxysporum Berk and Curt.	
Cunninghamella achinulata	
Alternaria circinans	
Alternaria tencissima	*A. tencissima*
Aspergillus flavus Link	
Aspergillus niger	*Aspergillus flavus*
Ascochyta mycosphaerella rabiei	

Fungi isolated from untreated seeds	Fungi isolated from treated seeds
Drechslera spp.	
Penicillium javanicum Van bemya.	
Rhizopus arrhisus Fischer	
Fusarium solani (Mart.)	
Fusarium semilectum Berk and Curt.	
Fusarium moniloforme Sheld.	
Cladosporium oxysporum Berk and Curt.	
Cunninghamella achinulata	
Alternaria circinans	
Alternaria tencissima	*A. tencissima*
Aspergillus flavus Link	
Aspergillus niger	*Aspergillus flavus*
Ascochyta mycosphaerella rabiei	

The result from isolation studies showed that in the case of linseed crop seeds, maximum association of *Fusarium* species was also observed on and in the treated and untreated seeds. The other fungi which were isolated from seed surface includes species of *Aspergillus, Alternaria, Curvularia, Chaetomium, Rhizopus, Macrophomina* and *Penicillium* while in treated seeds species of *Fusarium, Alternaria, Curvularia, Chaetomium* could be detected. Twelve species were internally seed borne and other externally seed borne.

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the isolates and planting them on sterilized moist blotter paper in Petridishes as well as planting them in pots filled with sterilized field soil. Suitable controls were also maintained.

Pathological studies revealed that *Fusarium solani* caused seed-rot, pod-rot and seedling rot. *Fusarium oxysporum* caused wilt, seed-rot and seedling rot of linseed. *Fusarium moniliforme* caused wilt and seedling rot of linseed. *Fusarium acuminatum* caused rot and wilt of linseed were also able to cause wilt, root-rot and seed-rot respectively.

In case of root-rot and wilt, infection appears to be seed-borne as the disease could also occur in infested soil.

In the present investigation, the initial pH of the medium drifted towards neutral to alkaline side. In highly alkaline media this was towards lesser alkalinity and in highly acidic media the drift was towards less acidic side. According to Lilly and Barnett (1951), "These changes in pH are due to changes in the relative amounts of the acids and bases formed or withdrawn and to the ionization constants of these compounds."

The population of all the three species of *Fusarium* declined in the soil samples amended with glucose and sodium nitrate mixture. It was also observed that as the C : N ratio was increased, the number of colonies declined more rapidly in the soils. The population of *F. equiseti* and *F. oxysporum* for all C : N ratios decreased in comparison with control upto the end of 4 weeks. The population of *F. solani* increased during the first week then declined upto the end of incubation period.

In a preliminary test, the efficiency of a number of fungicides viz., Aureofungin (a heptaene antibiotic), Benlate (Methyl) 1-

(butylearnamoyl) 2- benzimidazolecarbamate), Brassicol (Pentachloronitrobenzene) Cercobin (Benzene thiophanate), Difolatan (Cis-N-1(1,1,2,2-tetrachloroethyl) thio-4-cyclo-hexene-4, 2-dicarboximidie), *Ferbam* (ferric dimethyldithio-carbamate), *Plantvax* (DCMOD), 2, 3 dihydro-5 carboxanilido-6-methyl-1, oxathiin-4, 4 dioxide), thiram (tetramethyl thiuram disulphide or bis (dimethyl - thio - carbomoyl) disulphide) and Vitavax (DMOC, 5, 6-dihydro-2methul - 1, 4-oxathin-3-carboxanilide against three species of *Fusarium* was tried in vitro. The fungicides were added to the basal medium at different concentrations (50 - 1000 ppm) and flasks was steam sterilized for 3 successive days for 30 minutes.

The results show that Benlate at 50 ppm, Cercobin at 500 ppm, Difolatan 100 ppm, Plantvax at 500 ppm and Vitavax at 1000 ppm were found to be inhibitory to the growth of present organisms, whereas rest of the fungicides including the antibiotic. Aureofungin failed to inhibit the growth of present *Fusarium* species even at the concentration of 1000 ppm (maximum concentration taken). The concentrations of fungicides, which inhibited the growth of the organisms, were then employed to check the population of *Fusarium* species in soil. In

addition to this, Tecto '40' (Thiabendazole (42.8 percent), 2-(4-Thiazolyl) benzimidazole was also used.

All the fungicides were amended in the soil at a concentration, which was found to be inhibitory to the growth of the organisms in culture media. Tecto '40' was amended to the soil at a concentration of 500 ppm. The fungal inoculum was incorporated in the soil after 24 hours of addition of the fungicide.

Difolatan was found to have a marked retarding effect on the survival of all the three species of *Fusarium*. At a concentration of 100 ppm, the fungal survival was almost eliminated. The incidence of survival of *F. equiseti* and *F. solani* was slightly increased in case of Vitavax and Cercobin respectively as compared to other fungicides. Tecto '40' was quantitatively less effective than Benlate in reducing the population of present species of *Fusarium*. Similar results were also obtained by Ilyas *et al.* (1976) for *Macrophomina phaseolina*.

Temperature has markedly affected the population of *Fusarium* species in the present soils. The results clearly showed that when the temperature was high, i.e. during April, the number of colonies isolated

were less, being minimum in July might also be due to heavy rains which resulted in poor aeration of the soils.

Moisture, which is important for the growth of soil fungi, has a pronounced effect on their distribution. In water logged fields anaerobic conditions affect them adversely. Saxena (1955) has reported that under such conditions only those forms thrive which are adapted to aquatic conditions such as species of *Allomycee* etc., members of *saprolegniaceae* and *phythieceae*.

Percentage germination of present fungi was notably effected when placed in seed coat leachates and seed extracts of linseed. On the other hand in general a high percentage of spore germination of above fungi was recorded in sterilized double distilled water.

15 days old culture filtrates of *Fusarium oxysporum, Fusarium solani* and *Curvularia lunata* affected the seed germination of linseed. Concentrations of culture filtrates above 20 percent inhibited the germination of seeds considerably.

There was a marked effect of culture filtrates on the growth of seedlings. The culture filtrates almost checked the growth of seedlings,

when they were planted in pests. Their growth was not vigorous as compared to control plants. In another set of experiment in which plants (15 days old) were kept in culture filtrates (in 150 ml conical flask) under laboratory condition for 7 days. It was observed that roots became pale and in a few cases localised dark brown spots developed. While plants kept in sterilized distilled water, no change was observed and they remained healthy.

Seed extracts and seed-coat leachates of linseed exerted influence on the spore germination of all the dominant members of the seed mycoflora, but the magnitude of influence varied with the forms. Seed extract had the maximum effect (74.73 percent) reduction in spore germination. On the spore germination of *Cladosporium* followed by *Alternaria* spp. (53.30 percent), *Alternaria flavus* (49.70 percent) and *Rhizopus* spp. (49.20 percent). Seed coat leachates were comparatively less effective as it showed pronounced effect on the spore germination of only *Cladosporium spp.* and *Penicillium spp.* Other fungal forms were affected to a lesser degree by both extract and leachates and showed more than 50 percent spore germination in their presence.

On the basis of preliminary study, ten fungicides viz., *Agallol, Agrosan,* Benlate, Brassicol, *Captan,* Cercobin, Farbam, Hinosan, TBZ, and *Vitavax* were found effective. They were further tried on seeds of *Linum usitatissimum.* Three concentrations viz. 500, 1000 and 1500 ppm solution was prepared in sterilized distilled water in sterilised flasks and seeds were treated with each concentration.

Agallol, Agrosan-GN, Benlate, Ceresan, Cercubin, Farbam. Hinosan, TBZ and Vitavax completely inhibited the growth of all the pathogenic fusaria in the laboratory while Dithane Z-78, Difolatan, Thiram and Kirticopper, were unable to check their growth. Brissicol, Blitox-50 and Sulphur dust were also found effective against all the pathogens except *Fusarium oxysporum.* Captan was proved to be ineffective against *Fusarium solani* while Plantvax was unable to check the growth of *Fusarium equiseti.*

All the effective fungicides used for seed treatment reduced the fungal population as compared to untreated seeds (control). It was further noticed that use of these fungicides on seeds did not cause any harmful effect on their germinability.

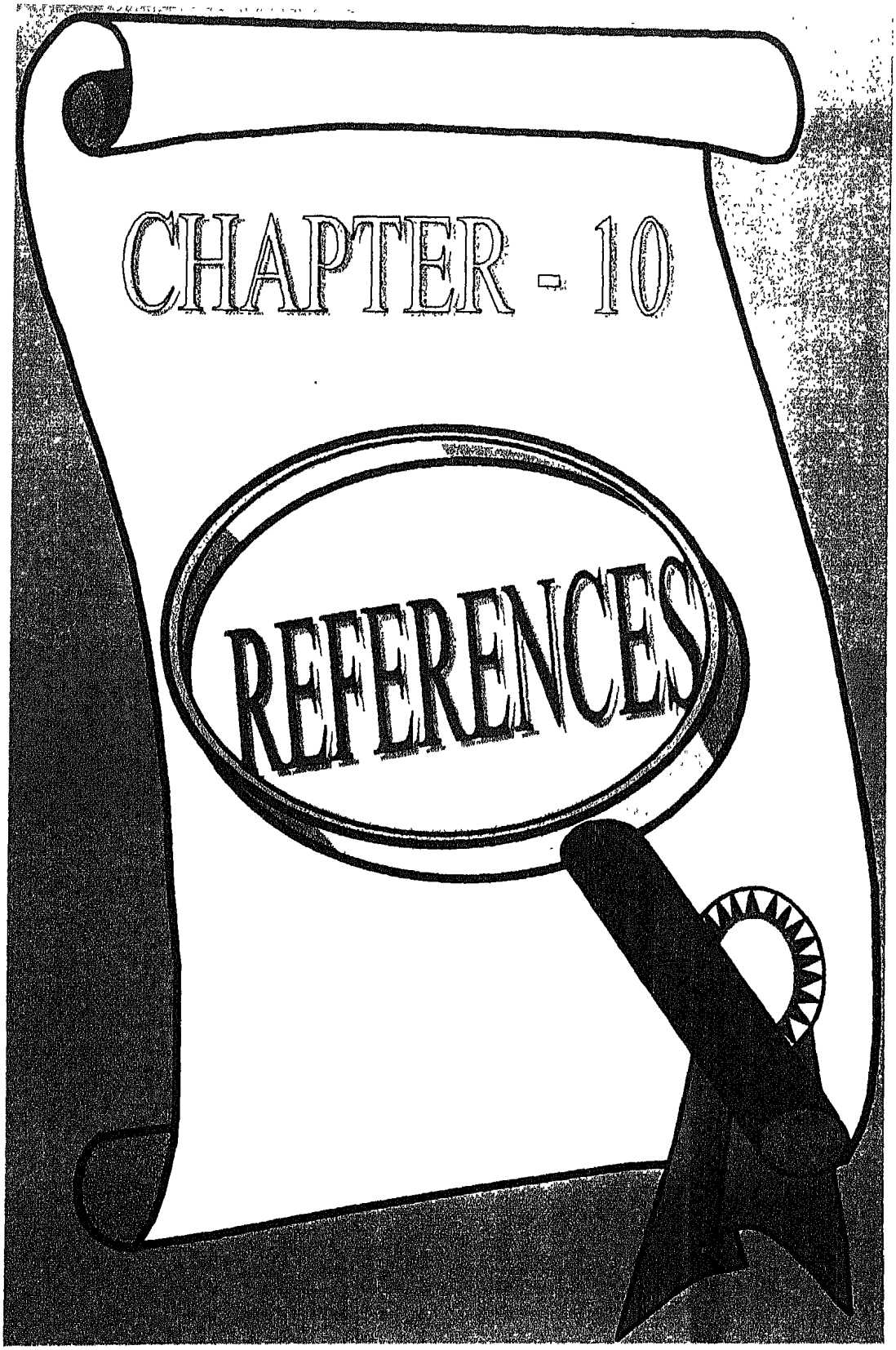
The present work deals with the effect of leaf extracts of a number of medicinal plants on the spore germination of the fungi under study. Spore suspensions were made in the supernatant extract at two concentrations viz. 100 percent and 50 percent.

Out of five medicinal plant tried, leaf extract of Neem (*Azadirachta indica*) at 100 percent concentration completely checked

the spore germination of all the three fungi, while 100 percent garlic bulb extract (*Allium sativum*) spores of *F. equiseti* could only germinate upto 20 percent. Next of Neem and Garlic extracts, Ocimum leaf extract (100 percent) exhibited anti-fungal property where spores of *F. solani, F. oxysporum* and *F. equiseti* could germinate upto 32, 18 and 42 percent respectively. In comparison to the above leaf extracts, spore germination of *F. solani, F. oxysporum* and *F. equiseti* was 92, 96 and 92 percent respectively in control sets (distilled water).

Results from the experiments clearly show that out of five high yielding varieties of linseed (*Linum usitatissimum* L.) crop tested against *Fusarium oxysporum, Fusarium solani* and *Fusarium equiseti,* N - 55 and Neelam were found resistant to the pathogens under investigation.

CHAPTER - 10



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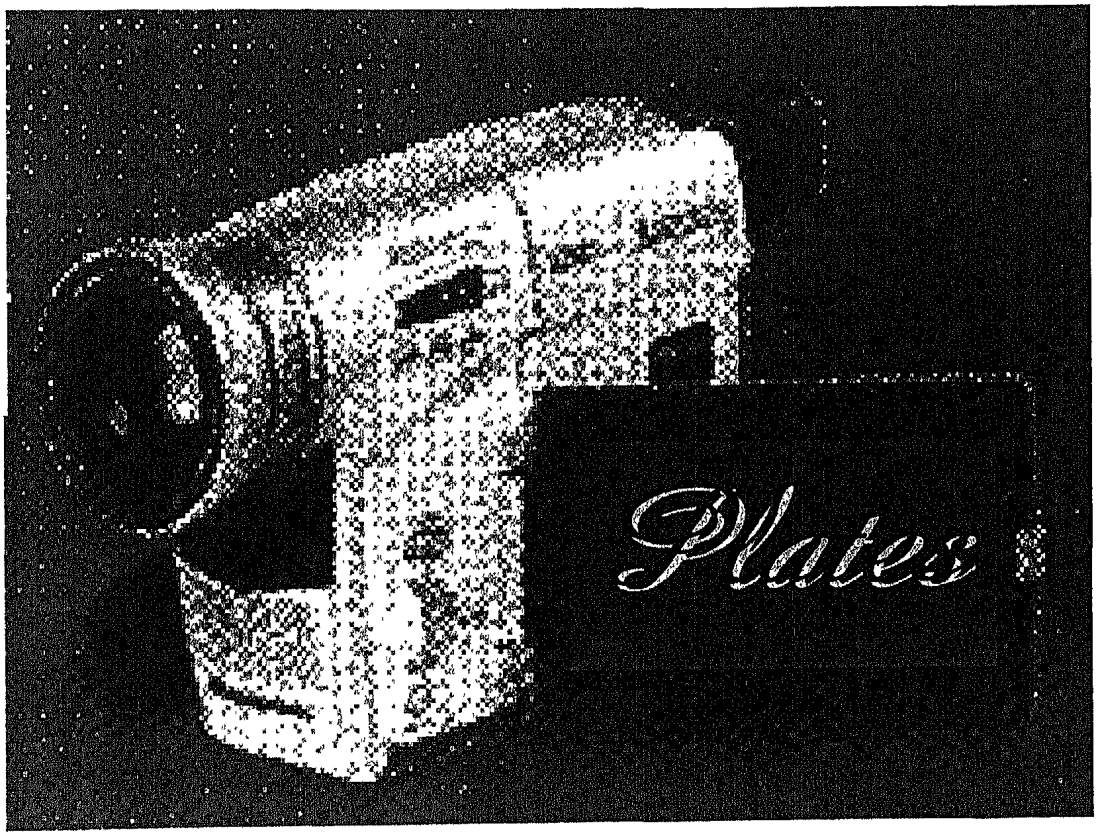
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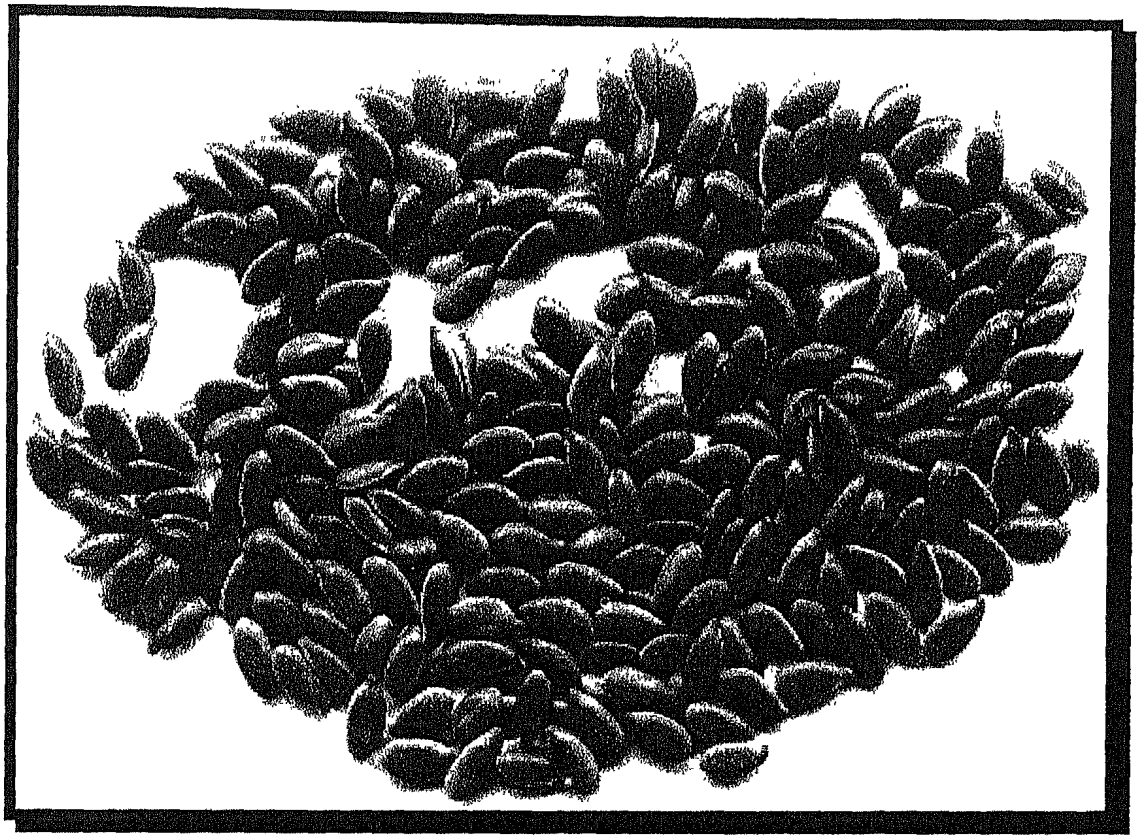
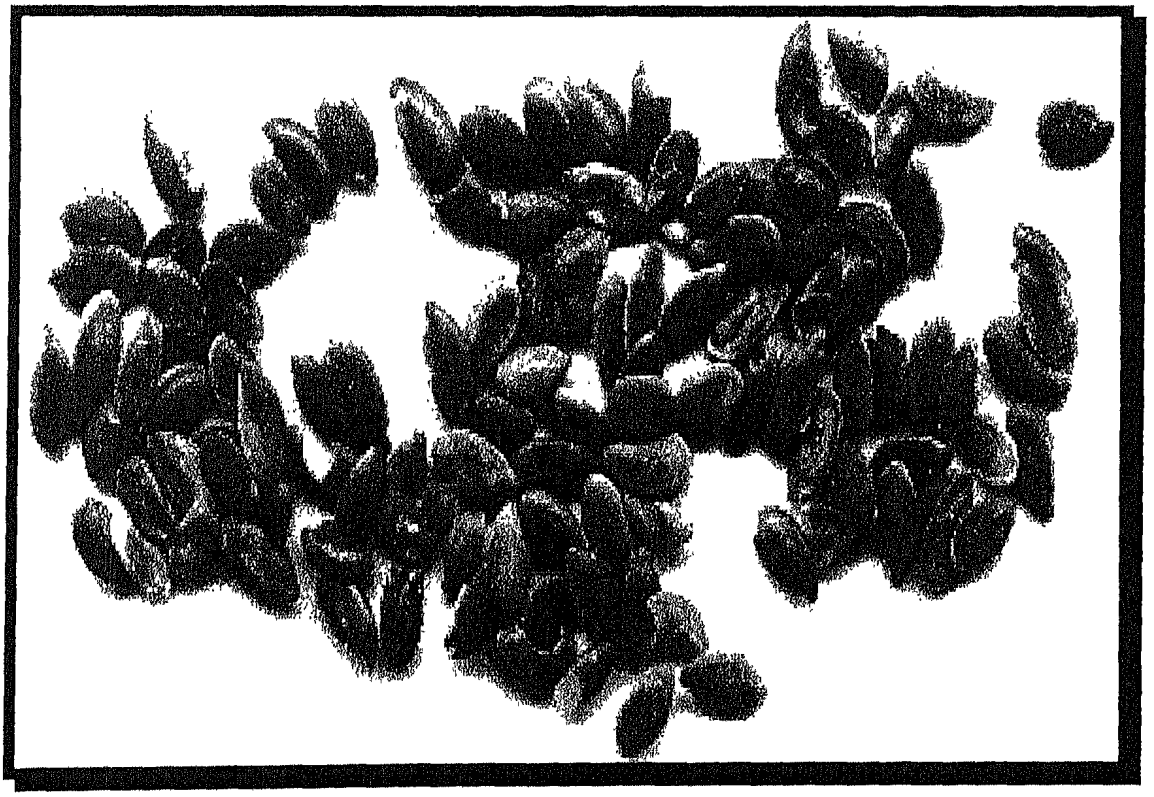
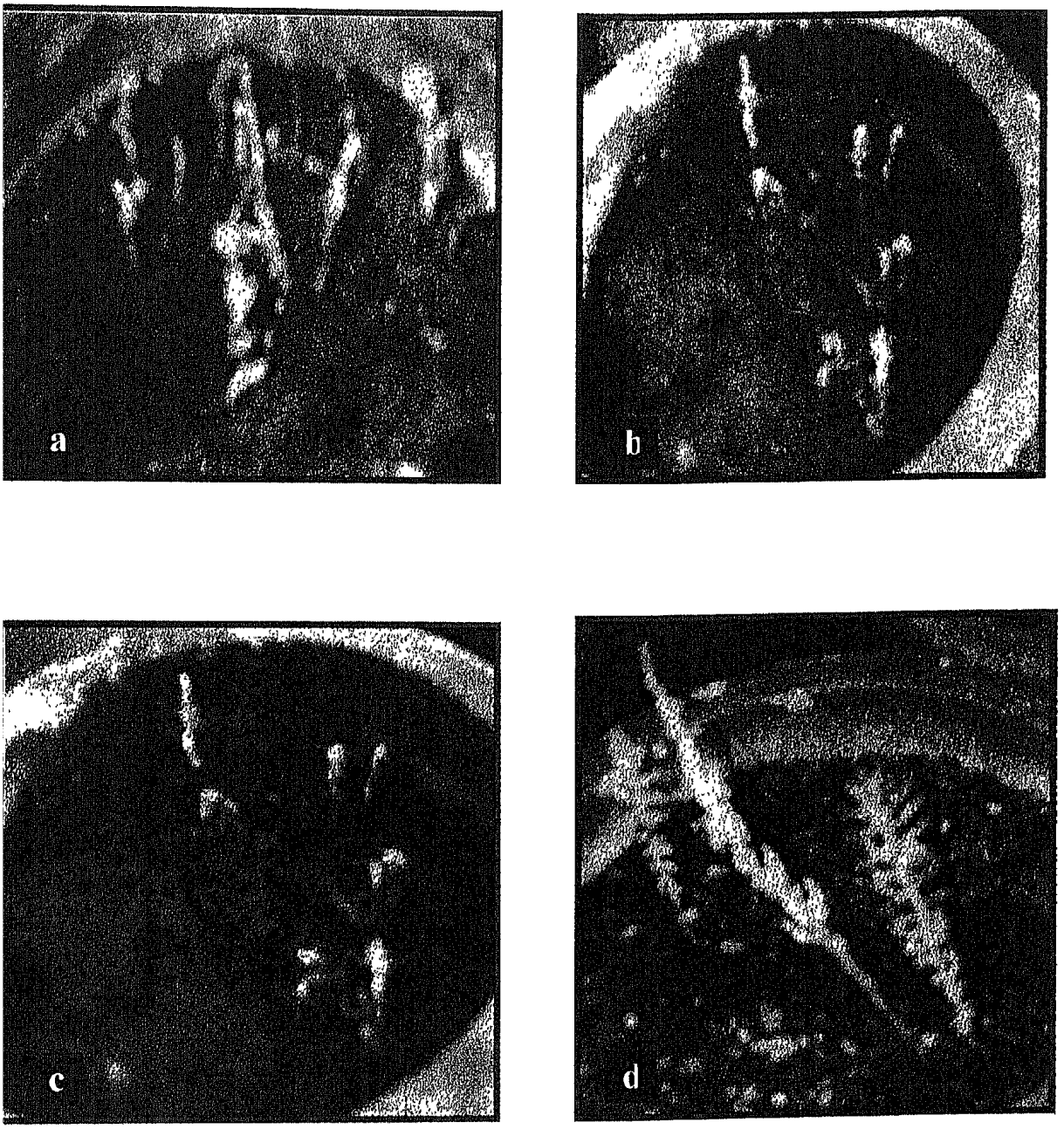


Healthy seeds of Linseed (*Linum usitatissimum* L.)



Defective seeds of Linseed (*Linum usitatissimum* L.)



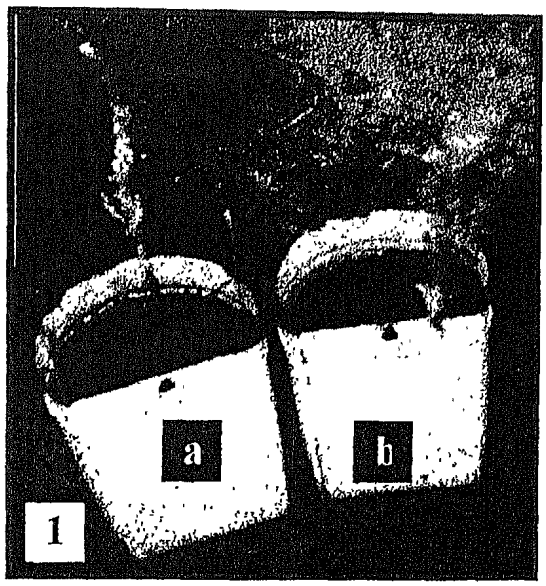
Fusarial wilt of Linseed (*Linum usitatissimum* L.) at seedling stage

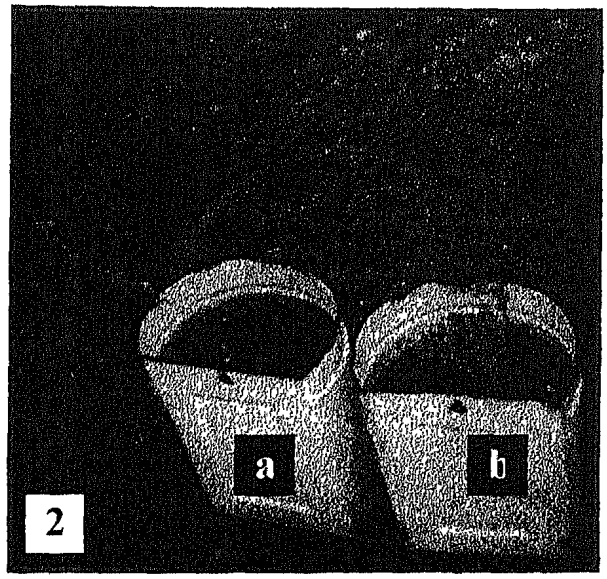
a. *Fusarium oxysporum*

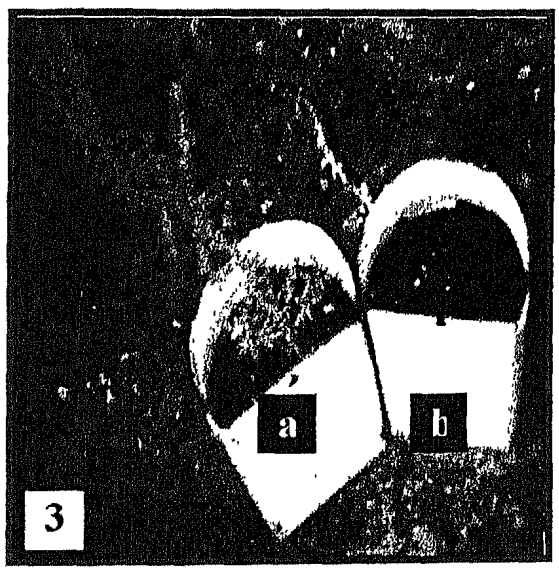
b. *Fusarium solani*

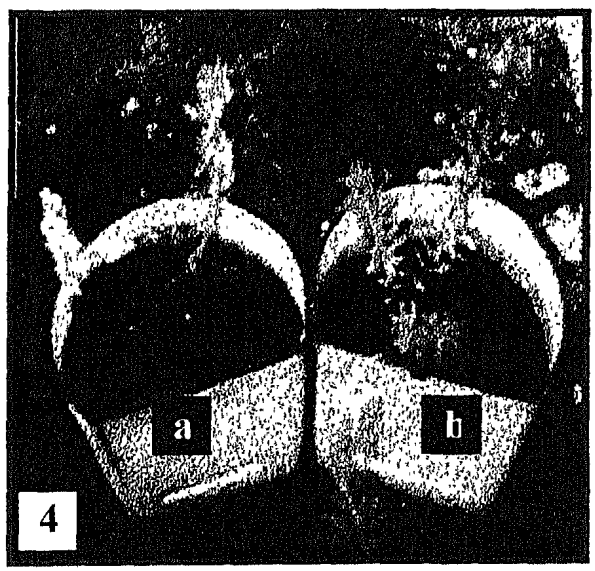
c. *Fusarium equiseti*

d. Control



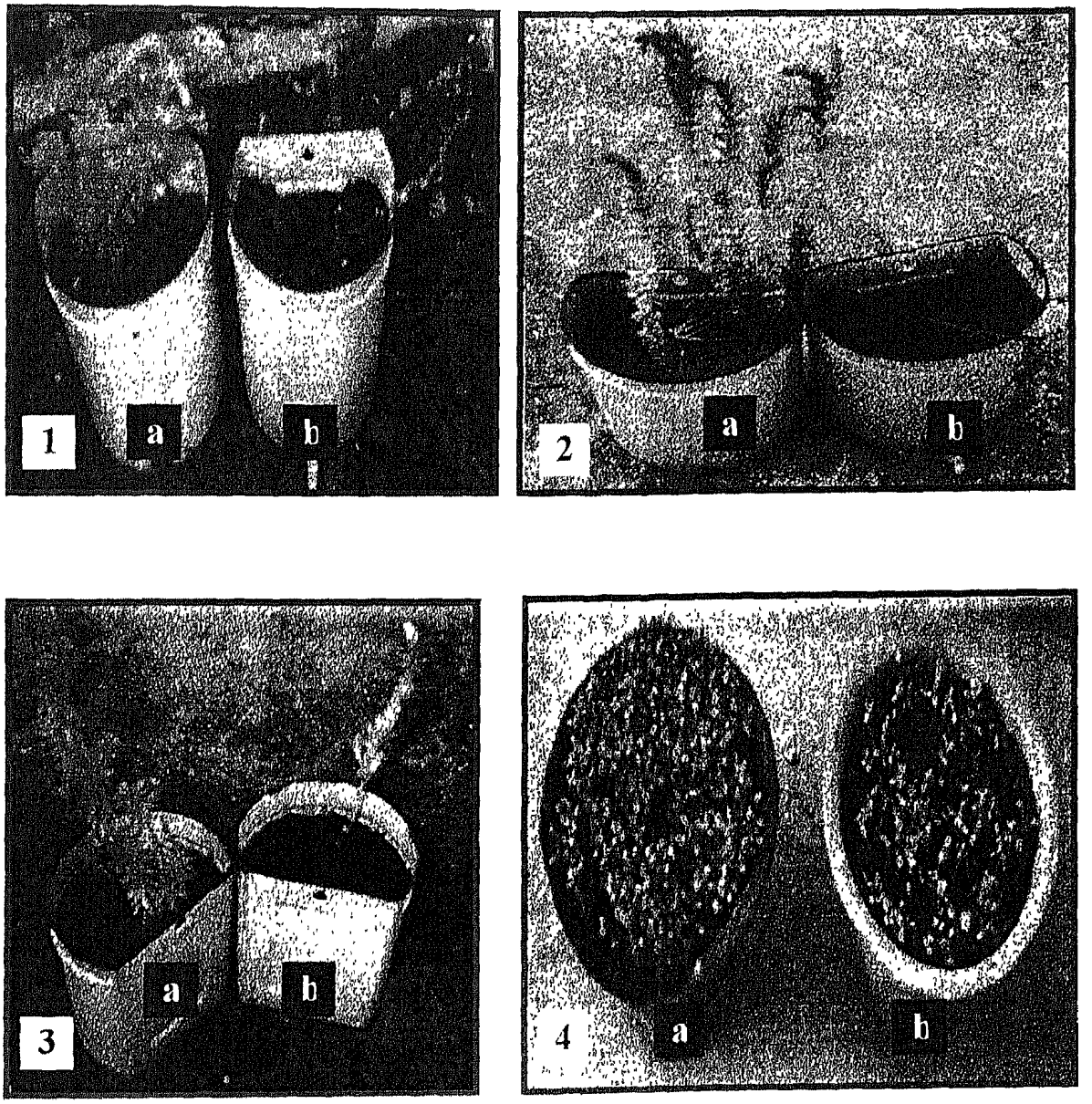






Fusarial wilt of Linseed (*Linum usitatissimum* L.) at seedling stage

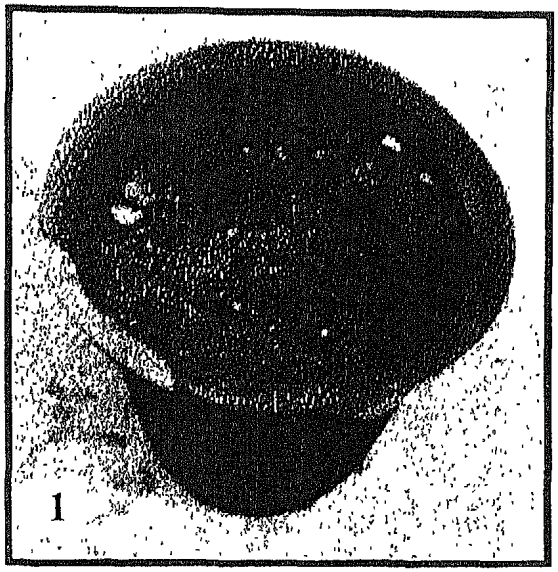
1.	a.	*Fusarium solani*	b.	Control
2.	a.	*Fusarium oxysporum*	b.	Control
3.	a.	Control	b.	*Fusarium equiseti*
4.	a.	*Fusarium equiseti*	b.	Control

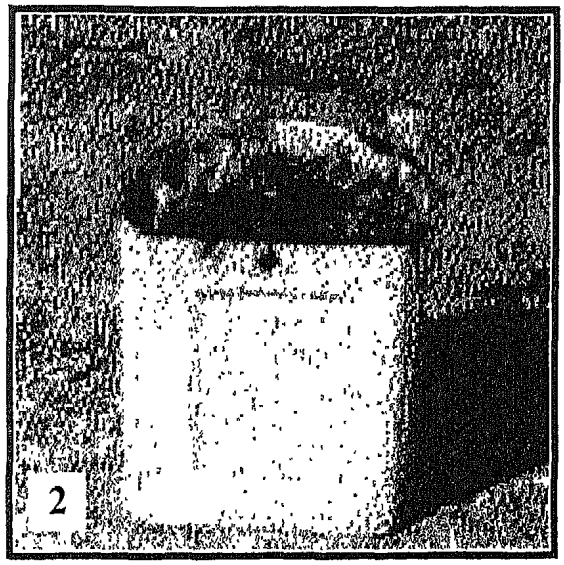


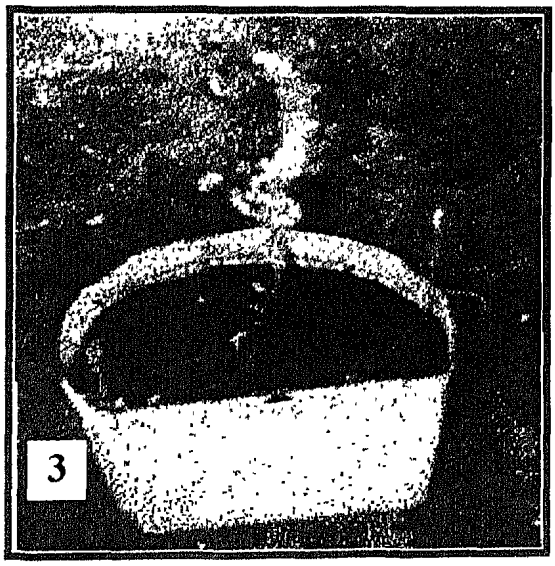
Wilted plants of Linseed (*Linum usitatissimum* L.)

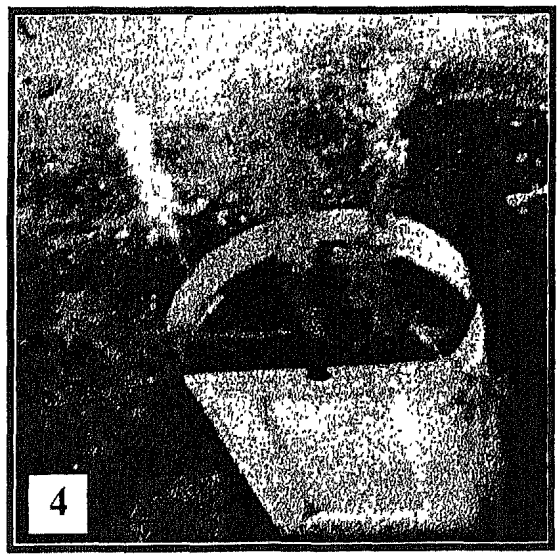
1. a. Control b. *Fusarium oxysporum*
2. a. Control b. *Fusarium solani*
3. a. Control b. *Fusarium oxysporum*
4. a. Control b. *Fusarium equiseti*

PLATE - 5



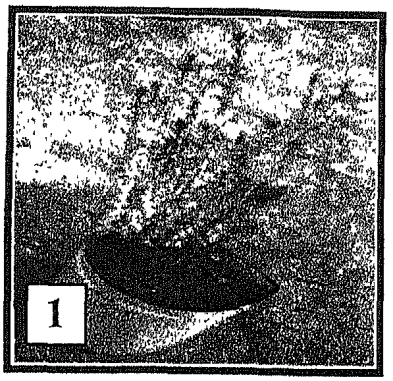


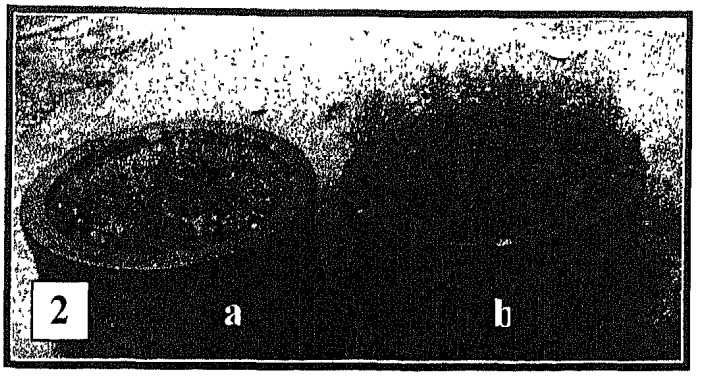


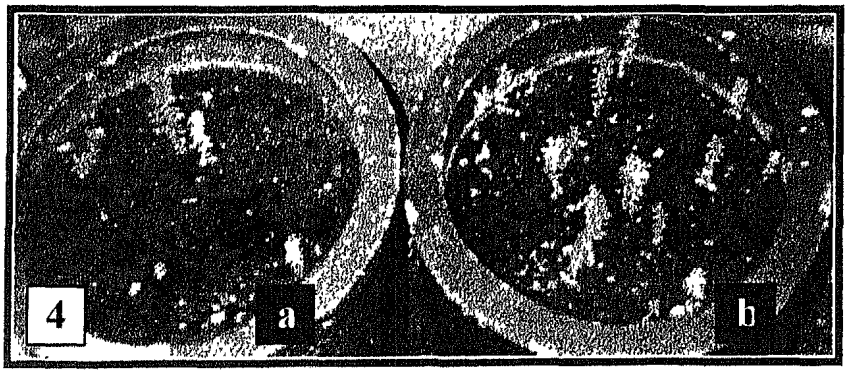


Wilted plants of Linseed (*Linum usitatissimum* L.)

1. *Fusarium oxysporum*
2. *Fusarium solani*
3. *Fusarium equiseti*
4. Control

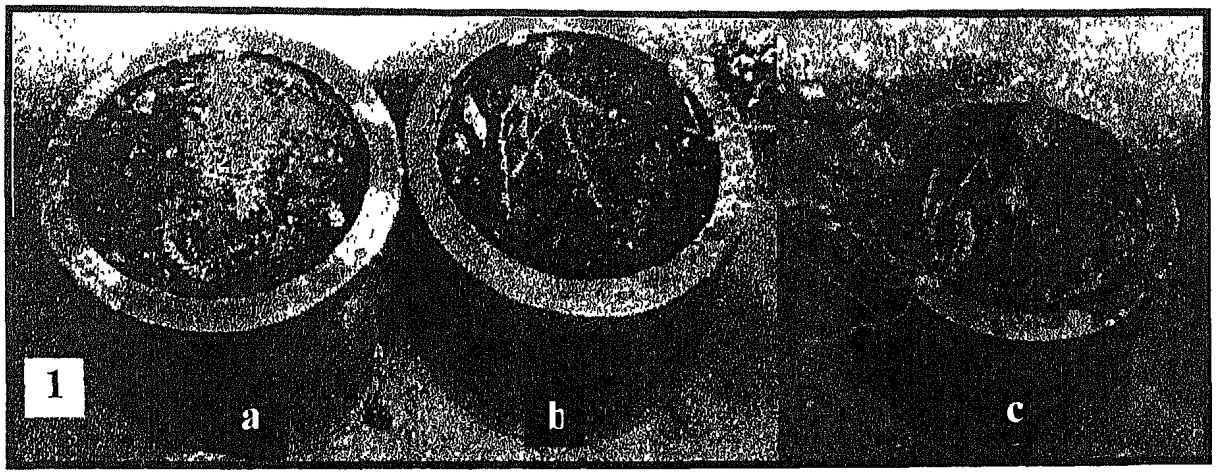


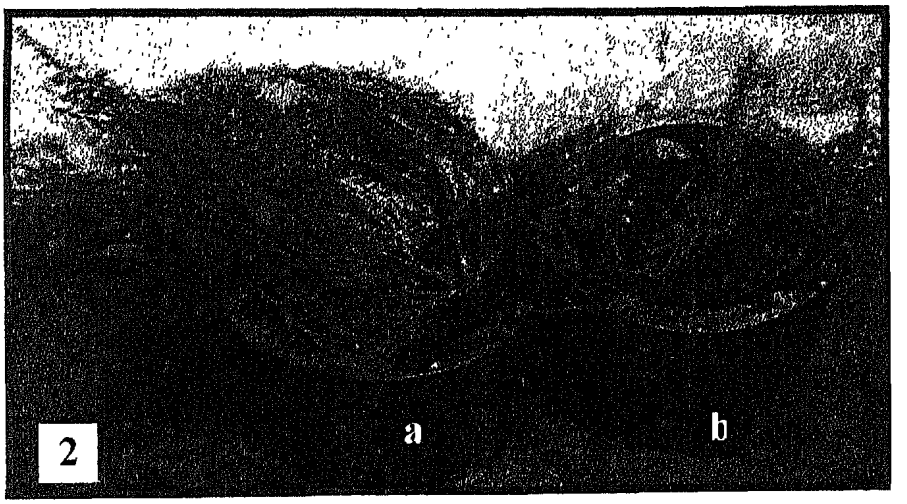


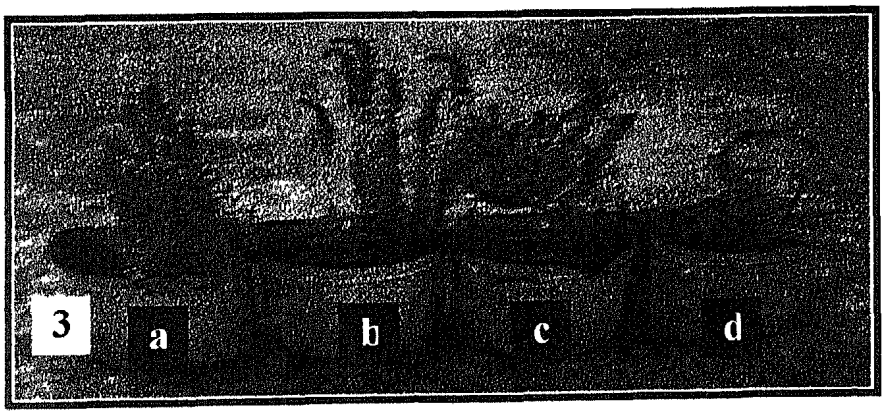


Wilted plants of Linseed (*Linum usitatissimum* L.)

1. *Fusarium oxysporum*
2. a. *Fusarium solani* b. Control
3. a. Control b. *Fusarium equiseti*
4. a. *Fusarium solani* b. Control

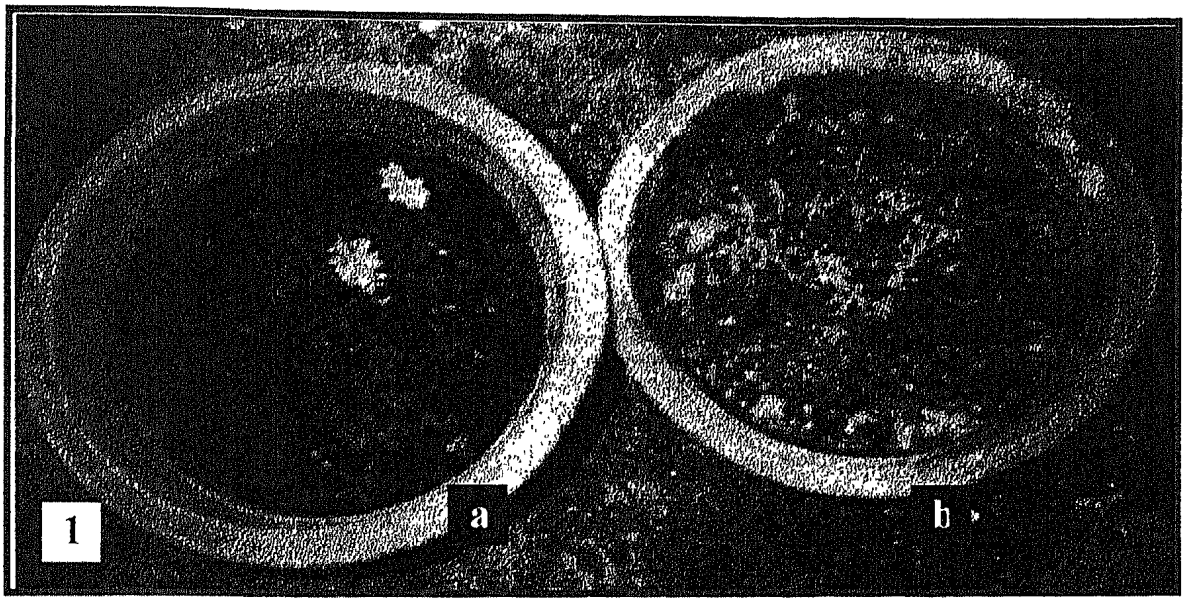


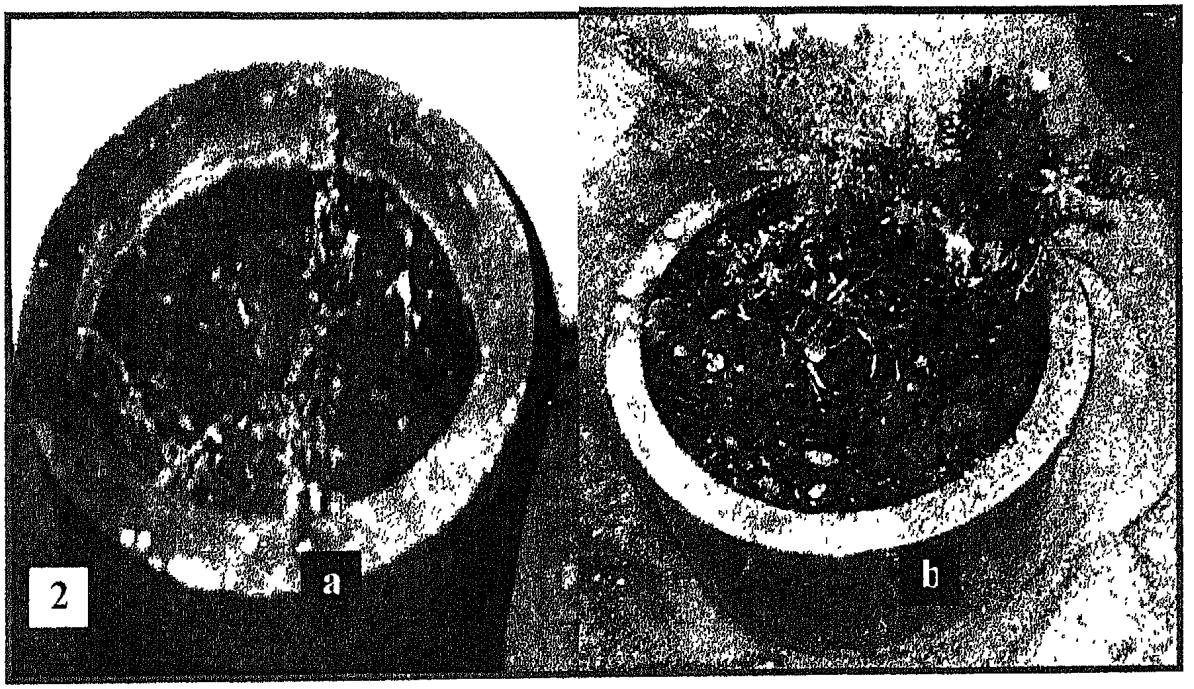




Wilted plants of Linseed (*Linum usitatissimum* L.)

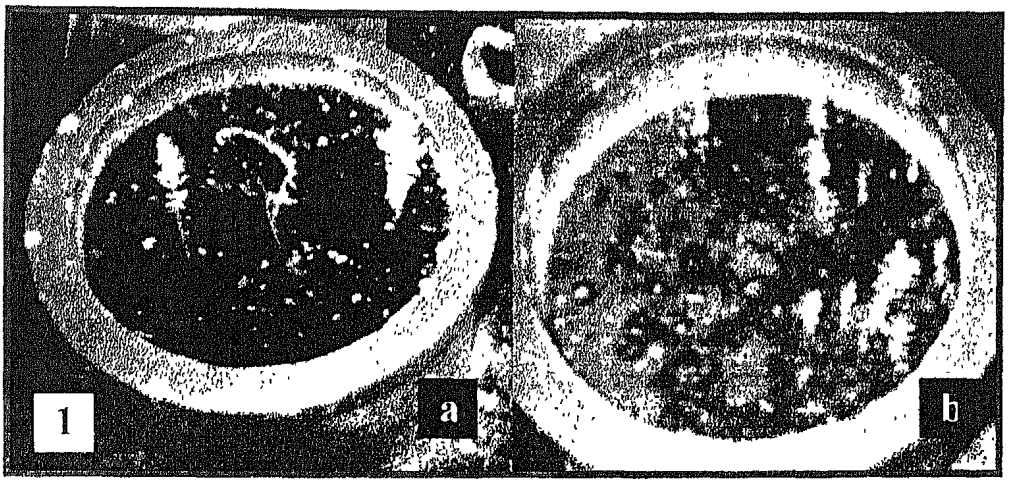
1. a. Control b. *Fusarium oxysporum* c. *Fusarium Solani*
2. a. Control b. *Fusarium solani*
3. a. Control b. *Fusarium solani* c. *Fusarium oxysporum*
   d. *Fusarium equiseti*

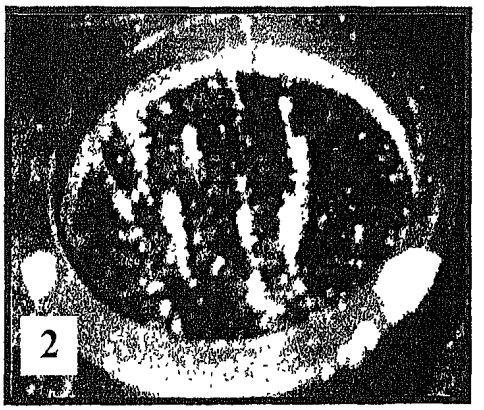


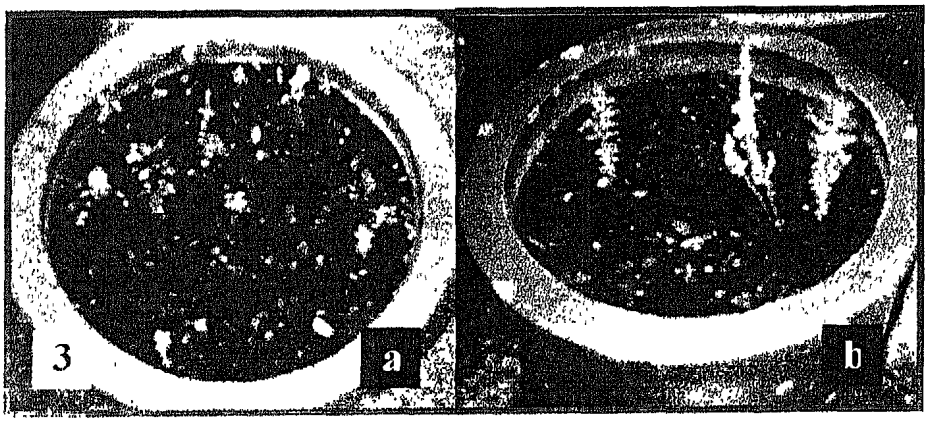


Fusarial wilt of Linseed (*Linum usitatissimum* L.)

1. a. *Fusarium oxysporum* b. Control
2. a. *Fusarium solani* b. Control







Fusarial wilt of Linseed (*Linum usitatissimum* L.)

1. a. Control b. *Fusarium oxysporum*
2. *Fusarium solani*
3. a. *Fusarium oxysporum* b. *Fusarium solani*